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CORTICIUM CENTRIFUGUM, A HETEROTHALLIC PATHOGENE OF APPLES[1]

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INTRODUCTION

In 1903 Eustace (*13*)[3] reported a previously undescribed rot of apples. This disease, formerly called Hypochnus rot (*9*), is termed "fisheye" rot in this paper. The rot was found in New York only, and on but two varieties of apples, Baldwin and Rhode Island. In each instance the decay followed scab and appeared as brown, sunken, circular lesions which varied in size. Isolations from the necrotic tissue yielded a fungus that was designated as *Hypochnus* sp. The pathogenicity of the fungus was demonstrated by inoculating apples of the same variety as those from which it was isolated, as well as 33 additional varieties and 5 varieties of pears. It was also ascertained that when infected fruit was held in moist chambers it gave rise to superficial delicate dirty-white mycelial mats.

As previously reported (*9*), the increasing prevalence of the so-called Hypochnus rot of apples has attracted attention on many eastern markets. Diagnoses based on the isolation and identification of the parasite have shown that the disease is more widespread than was formerly reported. On the markets the decay has been found on Baldwin, Rhode Island, Roxbury, and Ben Davis apples from New York; Winesap, Ben Davis, and Willow apples from southern Illinois; Winesap, Jonathan, and Rome apples from both Wenatchee and Yakima, Wash.; Jonathan and Winesap apples from Idaho; Yellow Newtown apples from Oregon; and Ben Davis apples from Virginia.

COMPARISON OF SYMPTOMS OF FISHEYE AND OTHER ROTS OF APPLES

On apples grown in New York the so-called Hypochnus or fisheye rot usually follows scab (pl. 1, A, C), although it has been found many times when no scab was discernible on the fruit (pl. 1, B). When following scab, it appears externally as a tan to brown circular area. (Pl. 1, A.) Individual lesions are usually less than 1 inch in diameter (pl. 1, A), but sometimes they coalesce to form larger areas (pl. 1, C). The injured area is generally markedly depressed. The

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[3] Reference is made by number (italic) to Literature Cited, p. 292.

depression and the color change clearly delimit the necrotic region from the normal tissues. The enlargement of the lesion is uniformly centrifugal, resulting in the maintenance of the point of inception, commonly a small scab lesion, in the center of the decayed area.

The lesions are cone shaped, and the depth is usually approximately equal to the diameter on the surface of the fruit. The subepidermal tissues are browned and disintegrated. The tissues are characteristically dry and somewhat stringy, giving to the lesion as a whole a dry, spongy consistency. When infected apples are held under sufficiently humid conditions, as in moist chambers, the mycelium spreads over the surface of the apples, radiates uniformly from the lesions, and forms a characteristic, delicate, appressed, white mycelial mat. (Pl. 1, E.)

Eustace (*13, p. 125*) pointed out the similarities in macroscopic appearance of this rot and the pink-mold rot caused by *Cephalothecium roseum*, and presented the differences as follows:

> On fruit affected with C. roseum there is usually a conspicuous white or pinkish growth of the fungus in the center of an affected spot; whereas this new fungus does not show at all conspicuously on the surface of a decayed spot until made to do so by artificial conditions. On fruit, C. roseum is a very shallow growing fungus, penetrating the tissue not much more than an eighth of an inch, while this new fungus grows much deeper and in its late stage extends to the core.

The mycelium of the fisheye-rot fungus has been seen growing superficially on barreled apples from the State of New York, in which there was a high degree of humidity. When apples upon which the mycelium was growing were removed from the humid atmosphere necessary for this external growth of the fungus, the mycelium quickly disappeared.

A somewhat analogous condition occurred in the Northwest in that the symptoms of the new rot agreed very closely with those of other rots of northwestern apples that have received much attention. To make this clear, a brief discussion of the field rots of northwestern apples is necessary.

The first apple rot having its inception in the orchards in the Northwest was reported in 1900 by Cordley (*10*), and was found to be very prevalent on northwestern apples inspected on eastern markets. The disease was named anthracnose or northwestern anthracnose (*Neofabraea malicorticis*). The outstanding characteristic of the apple rot is the tan center and dark border of the skin over the lesion. The disease is for the most part confined to the more humid sections of the Northwest.

About 1920 a rot of similar appearance was found on the markets on northwestern apples shipped from localities said to be free from anthracnose. Isolations from these specimens yielded a fungus that did not sporulate. Because of the similarity in appearance of the lesions to those caused by anthracnose and the obvious difficulties involved in classifying the sterile pathogene, the rot was called false anthracnose.

In 1925 Zeller and Childs (*47*) reported a new apple disease in the Northwest. The fruit-rot phase of this disease, which also has its inception in the orchards, was named bull's-eye rot. The symptoms on the fruit were found to be very similar to those of anthracnose and false anthracnose, and the rot was thought to be the same as the false

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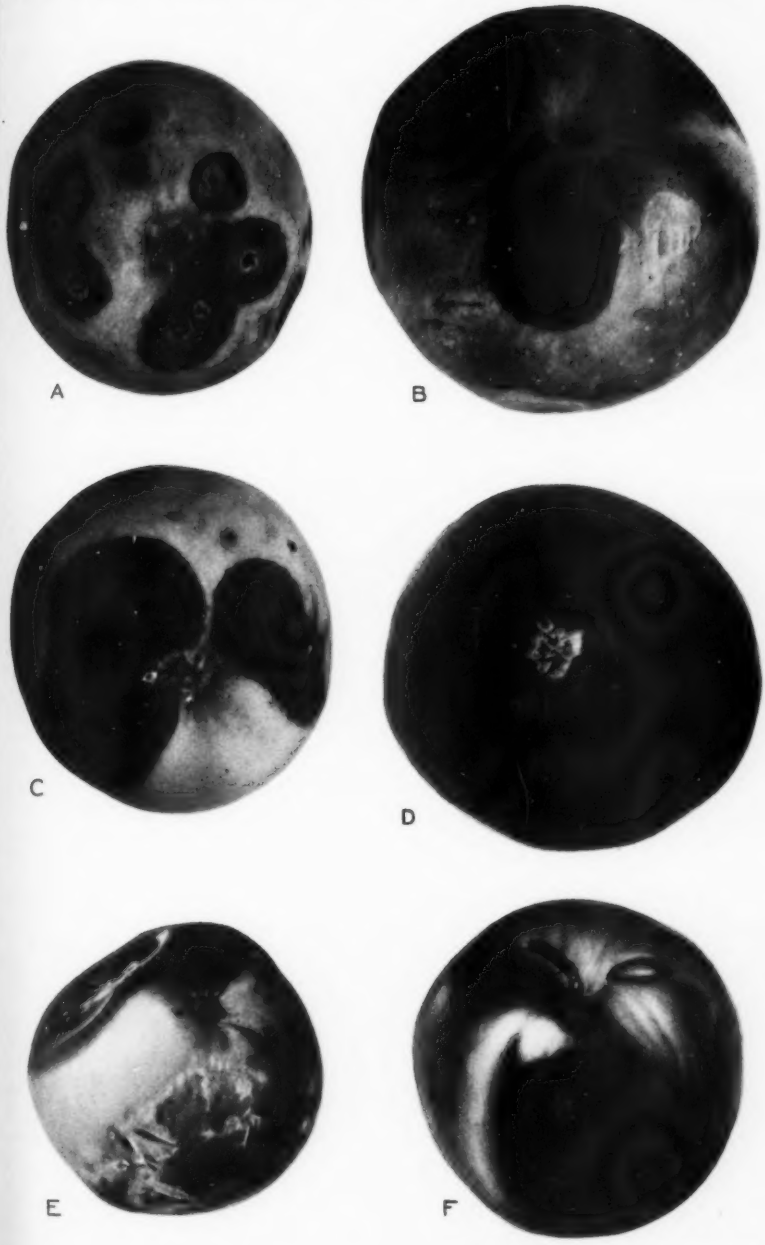

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Apples affected with fisheye rot caused by *Corticium centrifugum*

A–C, Baldwin: A, Small lesions following scab and responsible for name fisheye rot; B, large depressed lesion not following scab; C, large irregular lesion formed by coalescing of small lesions. D–F, Winesap: D, Small lesions, showing degree of depression; E, advanced stage, showing appearance of superficial mycelium after exposure to dry air; F, multiple infections, showing coalescence.

anthracnose reported on the markets. However, it is noteworthy that the false-anthracnose organism has been found to be consistently sterile, whereas the bull's-eye rot pathogene (*Gloeosporium perennans*) fruited readily.

In the process of making isolations for the purpose of diagnosing decays of northwestern apples found on eastern markets, each of the three organisms causing the three rots (anthracnose, false anthracnose, and bull's-eye rot) has been found many times. In addition to these three forms there was found, as reported by the writer (*9*), a fourth pathogene that had clamp connections and was shown by subsequent cultural studies to be morphologically identical in both its vegetative and fruiting stages with the nodose septate fungus previously isolated from eastern apples. The lesions from which these isolations were made were quite similar to those of the three rots already described on northwestern apples. As the causal organism of the new rot seemed identical with the pathogene of the apples from New York, it was felt that the same name should be used. As heretofore stated, the rot is most often found in apples from New York, following scab. The dark circular scab spot in the center, bordered by the tan depressed ring of discolored apple skin, presents a somewhat eyelike appearance. (Pl. 1, A.) Members of the trade on eastern markets had used the name fisheye rot to designate this type of lesion. Since the name already had some standing and is descriptive, it has been adopted and is being used as a common name for the disease.

It is seen, in summarizing, that four apple rots having the same general appearance have been reported on northwestern apples. The lesions consist of cone-shaped necrotic tissues. The point of the cone extends into the flesh of the fruit, and the base is formed by the discolored epidermis covering the lesion. The discolored epidermis is usually circular in shape, having a tan center and a dark border. (Pl. 1, D, F.) The color contrast between the central area and the border is often more pronounced on red, well-blushed surfaces than on yellow or lighter areas. The similarity between the four decays is so pronounced that in many instances isolations and microscopic studies of the fungi are necessary for diagnosis. However, some macroscopic differences have been observed. Bull's-eye rot and anthracnose are very similar. The necrotic tissues are moist and mealy; small lesions are not usually depressed; and superficial growth of the mycelium is very scanty and tufted, if present at all. Both fisheye rot and false anthracnose afford contrasts to these characteristics. In the latter two diseases the necrotic tissues are usually dry and spongy, the lesions even when very small are commonly depressed (pl. 1, D, F), and if the mycelium appears on the surface it is appressed and matted (pl. 1, E).

PREVALENCE OF FISHEYE AND OTHER ROTS OF APPLES

Any estimate of the relative prevalence of the four field rots found on apples grown in the Northwest is obviously confused by the difficulties of diagnosis. While it is felt that bull's-eye rot and anthracnose are much more prevalent in transit and storage, it is obvious that the greater publicity given these two diseases has somewhat obscured the presence and prevalence of both false anthracnose and fisheye rot.

Winesap apples from the Northwest showing the characteristic symptoms (pl. 1, D, E, F) were collected from a large number of fruit stores in late spring and cultured in the laboratory. These specimens showed almost 100 per cent false anthracnose and fisheye rot. In addition to this rather restricted investigation, many confirmatory isolations have been made in late March and April from apples mailed to Chicago by inspectors of the Bureau of Agricultural Economics, United States Department of Agriculture. This cultural work, together with observations made on some 10 or 12 other eastern markets during the last three years, indicates that both false anthracnose and fisheye rot are consistently present in northwestern apples in late spring. The percentage of decay in any given lot of fruit at this time of year is usually but not always small. The following quotation accompanied six Rome apples mailed to the writer for diagnosis:

> Car M. D. T. [Merchants Dispatch Transit Co.] 95826 out of Benton City, Wash., on October 17. In storage in Minnesota till March 21, arrived Detroit 25th or 26th of March. Shows decay ranging from 4 to 60 per cent, averaging 29 per cent, according to M. C. [Michigan Central] inspection records. Inspection restricted to top tier boxes and doorway.

Cultures made of these apples showed that both false anthracnose and fisheye rot were present. In fact, both diseases were found on one of the apples. The inspector of the car stated that the specimens sent to the laboratory were representative of all the decay found.

Inasmuch as pink-mold rot and the so-called Hypochnus rot, both found on apples grown in New York, are so similar in their macroscopic symptoms, and as pink-mold rot has received a great deal more attention, the importance of so-called Hypochnus rot has been overlooked. In the last three years approximately 100 cultures have been made from lesions on apples grown in New York that fitted the description of the rot as given by Eustace (*13*). In most of these cultures the fungus isolated fitted accurately, in both the vegetative and fruiting stages, his published description of the causal organism. In other cultures made from the same type of lesion, sometimes from the same apples, a macroscopically similar growth occurred. Microscopic examination, however, disclosed differences. Further treatment of this phase of the work is confined to later discussion in this paper. The morphological and pathogenic similarities are rather conclusive evidence that the pathogene isolated repeatedly from apples from New York inspected on many eastern markets and the fungus described by Eustace (*13*) are the same organism.

An examination of many cars of barreled apples from New York has shown as high as 25 per cent of the lot to be infected with fisheye rot. Diagnoses in these cases were confirmed by culturing representative specimens. It has been found that fisheye rot is much more common late in the storage season than at any earlier time.

METHOD OF INFECTION

Although very few data are at hand, the studies and observations that have been made indicate that the inception of the fisheye decay occurs in the orchards. The fungi comprising the group to which the form that causes fisheye rot belongs are, in the main, saprophytes that are found on stumps and dead branches. In the labora-

tory the causal fungus has grown well on apple twigs and stems. The writer found it growing over the surface of apples in an orchard near Wenatchee, Wash. The superficial growth was characteristic, and microscopic examination of the hyphae showed the clamp connections. It is considered quite probable that the organism establishes itself in tissues of the mature fruit that are either dead or at a low ebb of life, for example, stems, calyxes, or lenticels, in the orchard, and develops after the fruit is shipped. It has been found many times on the surface of eastern and western apples that were not decayed.

A study was made to test the assumption that the causal fungus was present in the stems of apples. Fifteen sound Winesap apples were selected from a car, inspected on the Chicago market, in which fisheye rot was found. The apples were not sterilized but were held in moist chambers in the laboratory for three weeks. In nine of the specimens the nodose septate fungus grew out from the stems and over the shoulders of the apples. In this and similar observations no decay resulted from the superficial mat. This is taken as evidence that an opening is necessary for infection. Eustace (*13*) obtained negative results when he placed inoculum on the surface of apples, and concluded that the fungus could not penetrate the sound epidermis. Temperature studies have shown that the decay does develop under commercial cold-storage conditions. It seems highly probable that the lesions found on fruit in the markets have resulted from an incipient infection that was present, but invisible, when the apples were packed. In brief, it is felt that the disease has its inception in the field and that it develops but does not spread in transit or storage.

CULTURAL STUDIES

From the isolations previously discussed, some 50 strains of the pathogene, considered representative of the different localities mentioned, have been carried in the laboratory. The studies of the cultures were made in a comparative way. Thus, although initial studies were made on strain No. 211–C, isolated from Baldwin apples grown in New York, the morphological and physiological characteristics that were determined for this form have been found to be duplicated by other strains from New York and from all other localities reported. Some few strains have shown differences in culture, but the variations are small and mostly of a physiological nature. Five cultures from New York apples differed slightly from the other New York forms. No differences between strains have been found to be correlated with the localities from which they came. Sufficient studies to determine whether all the forms isolated should be referred to the same species have not been completed. However, as will be more fully discussed later, it is felt that enough evidence has been obtained to justify the conclusion that the same species is present in all the different localities from which the diseased specimens were shipped. The present paper deals only with the similar strains found in the various localities; a discussion of the variants is postponed.

The hyphae were smooth and white and had clamp connections, commonly one at each septum. (Fig. 1, B, C, E.) The hymenium consisted of loosely interwoven hyphae identical in appearance with those shown in the drawing of Eustace (*13, pl. 2*). The clavate

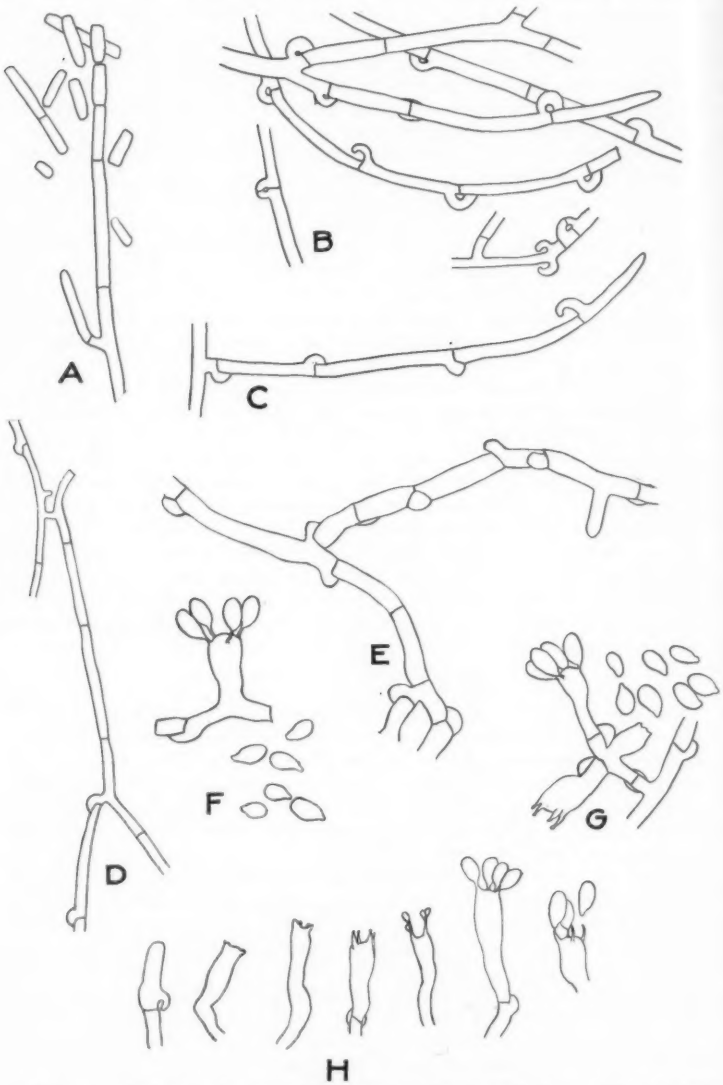

FIGURE 1.—Mycelia and spores from different strains of *Corticium centrifugum;* × 700: A, Haploid mycelium and oidia of single-spore strain No. 14; B, hyphae showing irregular and incomplete clamps in the mycelium over the inoculum of single-spore strain No. 14, six days after crossing with No. 266–A (fig. 2, A); C, hypha showing normal and incomplete clamps 2 cm. behind the inoculum of strain No. 14 in the new mycelium that had developed six days after crossing with No. 266–A (fig. 2, A); D, anastomosis of hyphae present at place of contact between strains Nos. 274 and 2 (fig. 2, B); E, hyphae from perfect strain No. 211–C, isolated from apples grown in New York; F, basidium and spores from No. 211–C; G, portion of hymenium and spores from perfect strain produced by crossing two sterile western strains (Nos. 266–A and 274); H, stages in the development of basidia, sterigmata, and spores

basidia, arising directly from the hyphae and bearing four spores on sterigmata (fig. 1, F, G, H), agree in manner of origin and development as well as in form and size with the previously published characterization. Further agreement is found in the length and number of the sterigmata and the number, shape, and size of the spores borne at the distal extremities of the basidia. (Fig. 1, F, G, H.)

The pathogene grows very well on all the common culture media. It also grows well on apple twigs, apple stems, and bean pods. Spore formation has been more copious on bean pods and string-bean agar than on any other substrate tried. While some success has attended efforts to induce fruiting, enough failures have occurred to prevent the assumption that sporulation may be produced at will. The pronounced sensitivity of the vegetative phase to changes in humidity and moisture content of the medium, and other observations as well, indicate that the degree and rate of desiccation to which the fungus is subjected are important factors in sporulation.

Although temperature studies have not been completed, it has been found that on Baldwin apples from New York and Winesap apples from Washington an appreciable growth occurs at cold-storage temperature (32° F.). Apples were inoculated subepidermally with small portions of mycelium and placed in a commercial cold-storage room the same day. The final observations made six weeks later showed that the pathogene had established itself and produced lesions averaging 15 to 20 mm. in diameter. The optimum temperature for growth on media lies between 65° and 75°. The maximum temperature for growth has not been determined, though slow growth occurred in Petri dishes on corn-meal agar at 90°. Inasmuch as humidity controls and accurate temperatures were not available, except in the commercial cold-storage room, the temperature studies were postponed.

Single-spore cultures of the fungus were made as follows: Portions of the hymenium of a fruiting culture that had been made from a Baldwin apple from New York and had been carried in the laboratory for two years were transferred under aseptic conditions to tubes of sterile water, shaken to remove spores, and further dilutions made. The spore dilutions were poured over thin layers of clear agar in Petri dishes. After the water had been absorbed so that the spores were lodged on the agar, the Petri dishes were inverted on the stage of a microscope for examination. When an isolated spore was found its location was marked with a circle of india ink drawn on the bottom of the Petri dish. Next, the agar within this area and the adhering spore were transferred to another dish containing a thin layer of clear agar. The transferred spores were then examined macroscopically at intervals until after germination to make sure that each colony was from a single spore. When this had been ascertained, transfers were made to test tubes of agar and the strains were numbered. In this manner 70 single-spore cultures were made. While the mycelium of most of the single-spore cultures was microscopically similar to the parent culture, microscopic examination disclosed differences. The hyphae were smooth and white but had ordinary septa instead of clamp connections. None of the cultures formed either basidia or spores, but oidia were formed copiously by segmentation of hyphae. (Fig. 1, A.) Oidia have not been found in any of the strains that had clamp connections and formed basidia and spores, but have been found in all single-spore cultures.

Cultural studies reveal a variation in vigor among the different single-spore strains. Some are almost as vigorous as the parent strain, though the perfect or parent strain is on the whole more vigorous than any of its sterile progeny. One measure of the relative vigor in the different single-spore strains is shown by the rate of growth. Table 1 shows the results of a comparative study. Bean agar contained in Petri dishes was inoculated with the parent culture, each of its single-spore strains, and the two western sterile strains (Nos. 266–A and 274), respectively, and kept at room temperature. The cultures were allowed to grow two days to establish themselves before initial measurements were taken. The total radial increase and average daily increase during the next five days were recorded.

TABLE 1.—*Comparative growth rates of a perfect strain (No. 211–C) and of sterile strains of the fisheye-rot fungus*

Culture No.	Genotype	Growth in 5 days (mm.)		Culture No.	Genotype	Growth in 5 days (mm.)	
		Total	Average			Total	Average
211–C	*AaBb*	32.5	6.4	9	*Ab*	12	2.4
1	*AB*	24	4.8	10	do	5	1.0
6	do	5	1.0	14	do	14	2.8
15	do	8	1.6	16	do	13	2.6
18	do	5	1.0	21	do	14	2.8
23	do	13	2.6	27	do	5	1.0
4	*ab*	7	1.4	28	do	10	2.0
12	do	18	3.6	2	*aB*	4	.8
13	do	10	2.0	3	do	3	.6
17	do	7	1.4	7	do	10	2.0
20	do	12	2.4	8	do	18	3.6
22	do	5	1.0	11	do	10	2.8
25	do	(a)	(a)	19	do	5	1.0
26	do	4	.8	24	do	12	2.0
29	do	3	.6	266–A	*aB*	4	.4
30	do	3	.6	274	*Ab*	5	1.0
5	*Ab*	13	2.6				

[a] Died.

The parent culture, No. 211–C, grew more rapidly than any of its progeny or than the two western sterile strains. Duplicate plates showed that the variation in rate of growth is consistent for the individual strains. It will be noted, however, that the rate of growth does not vary with the genotype. When two strongly mating haplonts are paired the spread of the resultant colony proceeds at a faster rate after the diploid condition is initiated than it did while its two components were still in the haploid state. An example is seen in a comparative study of the amount of new growth in two colonies shown in Figure 2, A, 2 and 14. The faster rate of growth seems correlated with the perfect or diploid condition.

While the longevity of the fungus in culture has not been studied, it has been found that the diploid mycelium can live as long as six months without transference, though many cultures of the single-spore strains die in a few weeks if not transferred to fresh culture medium. Observations indicate that there is a correlation between mortality and the sugar content and moisture of the substrate.

The absence of clamp connections in the hyphae of the single-spore cultures indicated that the fungus was heterothallic. Pairing of the monosporous strains was therefore begun.

RESULTS OF CROSSINGS

CROSSING OF SINGLE-SPORE CULTURES OF FISHEYE-ROT ORGANISM

Thirty single-spore cultures of the fisheye-rot organism were selected arbitrarily for crossing studies. The cultures were numbered 1 to 30 consecutively. Culture No. 1 was paired with each of the 29 other cultures. The pairings were made on bean agar in test tubes by placing small pieces of inoculum of the two strains close together in each tube. Checks were made by transferring each of the 30 strains individually to other tubes. Microscopic examination of the cultures two weeks later showed that the crossing of six of the strains—Nos. 4, 12, 13, 17, 22, and 25—had resulted in clamp formation, but that no clamps were found in any of the other tubes nor in any of the checks. Basidia and spores were found in each of the cultures that had formed clamps, but they were not found in any of the other cultures. This is in keeping with the findings of others.

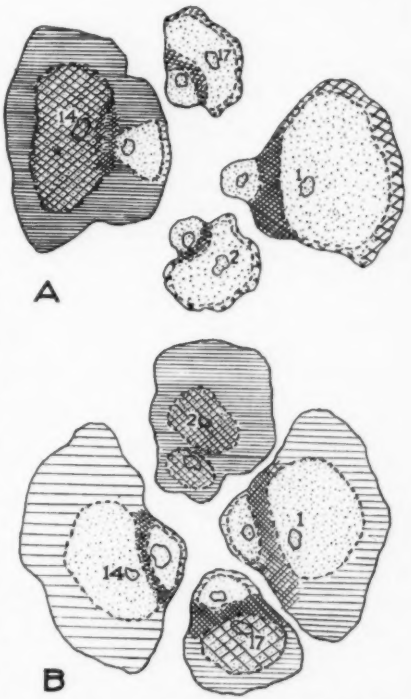

FIGURE 2.—Diagrams showing areas of clamp formation in crosses between two sterile western strains (Nos. 266–A and 274) and four sterile eastern strains (Nos. 1, 2, 14, and 17). The numeral on each colony is the number of the eastern strain with which the western strain was crossed. (Table 5.) Shading with horizontal lines denotes normal number of clamps; crosshatching denotes normal and irregular clamps; dotted areas denote oidia present; broken lines mark separation of old growth from that developed after crossing. In each cross the inoculum of the western strain is nearest the center of the diagram. A, Crosses of No. 266–A with the eastern strains. B, Crosses of No. 274 with the eastern strains

Since Bensaude (*1*) demonstrated heterothallism in the Hymenomycetes in 1918 by her work on *Coprinus fimetarius*, a comprehensive study of the condition has been made by many workers. In such studies definite criteria by which the haplonts and diplonts may be distinguished are essential. Clamp connections have become the accepted criterion in distinguishing between the haploid and the diploid condition. The monosporous mycelia of heterothallic Hymenomycetes has been found to be uninucleate and devoid of clamps, whereas the pairing of compatible monosporous strains results in a mycelium with paired nuclei that has clamps (*1*, *20*). Heterothallism is therefore evidenced by the presence of clamps in the compound mycelium and the absence of clamps in the monosporous mycelium. Cytological studies of heterothallic forms (*1*, *25*) have demonstrated that two monosporous uninucleate mycelia, by anastomosing, give rise to the compound mycelium with paired nuclei. In many Hymeno-

mycetes the haplonts form oidia but the diplonts do not. However, some variation has been found in this, so it is not always a reliable criterion. Although fruiting is usually confined to the binucleate phase, Kniep (*19*) has found that haplonts of *Schizophyllum commune* produced fruiting bodies and spores morphologically similar to the fructifications of diplonts, though all the spores produced on the sporocarp were of one sex. Similar findings have been reported by Kniep (*20*), Vandendries (*43*), Brunswik (*2*), Zattler (*46*), and Hanna (*17*). Therefore in the work presented here, although the formation of oidia and basidiospores has been taken into consideration, the basic criterion has been the presence or absence of clamps.

Inasmuch as culture No. 1 grew more vigorously than any of those with which it had mated, it was designated as a plus strain and those that had crossed with it were recorded as minus strains. Culture No. 1 and culture No. 17, the latter one of the minus strains, were then paired with each of the 28 other single-spore cultures in the manner previously described. Checks of each culture were made as before. Examination three weeks later proved that culture No. 1 had again mated with each of the six minus strains. Clamps were also found in the crosses between culture No. 1 and cultures Nos. 20, 29, and 30, which were therefore recorded as three additional minus strains. Since clamps were present in the crosses between culture No. 17 and cultures Nos. 1, 6, 18, and 23, these two sets of pairings thus resulted in the segregation of four plus strains and nine minus strains. No clamps were found in any of the other tubes nor in the checks.

It had been necessary to duplicate some of the pairings because the cultures made as checks did not grow. The failure of these strains to grow in the paired cultures would doubtless be overlooked because the two pieces of inoculum were placed in contact with each other and the mycelium from the more vigorous of two included strains could easily cover the inoculum of the other strain.

Consequently, a new technic was employed, and pairing between cultures Nos. 1 and 17 and each of the other single-spore strains was repeated in the following manner: Inoculations were made on sterilized string-bean pods, two pods, one much shorter than the other, being placed in each test tube. A few cubic centimeters of distilled water was added to each tube to prevent too rapid desiccation. In all inoculations with cultures Nos. 1 and 17 these strains were placed on the upper end of the short bean pod and the strain with which they were paired was placed on the upper end of the long pod. The cavities at the tops of the broken pods afforded a convenient receptacle for the inoculum. Care was exercised in the inoculation to insure the presence of but one of the strains on each pod. The distance separating the two pieces of inoculum thus afforded ample opportunity for checking the viability and growth of the less vigorous strains before the mycelium from the vigorous strains had developed far enough to cover them.

Examinations for clamps were made a week later, when in all tubes the mycelium of both strains had reached the lower ends of the bean pods and formed a profuse mat at the point of contact. Mounts for study were made from the bean tissue at the region of contact. Clamps were found in all crosses in which they had occurred previously and in two additional ones. Culture No. 15 crossed with No. 17, and

No. 26 with No. 1. All subsequent studies were made on bean pods as described.

To check the possibility that clamps might have been overlooked in the hyphae in any of the previous work, cultures Nos. 1 and 17 were twice paired with each of the remaining 15 strains that had not mated with either of them. In both series all results were negative.

The possibility that the ability of single-spore cultures to cross decreased with time, when carried in culture, was checked by pairing cultures Nos. 1 and 17 again with each of the 28 other single-spore cultures. When these tubes were examined clamps were found in each cross in which they had been found before, but no additional pairs showed clamps, indicating that no change in ability to cross had occurred.

The 15 unmated strains were then paired with one another after the well-known checkerboard method. Subsequent examination showed that the 15 strains divided into two groups of 7 and 8 individuals, repectively. Each individual of these groups formed clamps when paired with any individual from the opposite group but failed to form them when paired with another individual of its own group. Former failure to obtain positive results with all pairings was thus found to be due to the fact that there are four instead of two types of strains present. No morphological or physiological difference (Table 1) between strains had been observed to indicate this genotypic difference.

The necessary transfers were next made to complete a checkerboard pairing of all of the 30 single-spore cultures. The results of all possible pairings of the 30 single-spore cultures are shown in Table 2.

TABLE 2.—*Results of all possible pairings of 30 single-spore strains derived from a single hymenium of culture No. 211–C of Corticium centrifugum*

[− indicates absence of clamps; + indicates presence of clamps]

Mendelian interpretation	Culture	*AB*					*Ab*									*ab*										*aB*									
		No. 1	No. 6	No. 15	No. 18	No. 23	No. 5	No. 9	No. 10	No. 14	No. 16	No. 21	No. 27	No. 28	No. 4	No. 12	No. 13	No. 17	No. 20	No. 22	No. 25	No. 26	No. 29	No. 30	No. 2	No. 3	No. 7	No. 8	No. 11	No. 19	No. 24	No. 1	No. 6	No. 15	No. 18	No. 23
ab	No. 4	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	No. 12	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	No. 13	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	No. 17	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	No. 20	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	No. 22	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	No. 25	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	No. 26	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	No. 29	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	No. 30	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
aB	No. 2	−	−	−	−	−	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	No. 3	−	−	−	−	−	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	No. 7	−	−	−	−	−	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	No. 8	−	−	−	−	−	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	No. 11	−	−	−	−	−	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	No. 19	−	−	−	−	−	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	No. 24	−	−	−	−	−	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
AB	No. 1	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−
	No. 6	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−
	No. 15	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−
	No. 18	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−
	No. 23	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−
Ab	No. 5	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+
	No. 9	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+
	No. 10	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+
	No. 14	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+
	No. 16	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+
	No. 21	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+
	No. 27	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+
	No. 28	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+

It is seen that the progenies of the nodose septate form are differentiated into four types as demonstrated by their ability to fuse and form clamps. Kniep (*23*) listed 15 species of Hymenomycetes, among them *Corticium serum*, for which this phenomenon has been reported. This occurrence of four types of spores has been variously explained. In general it is interpreted as a sex phenomenon. Prell (*34*), however, favored the view that this and similar phenomena in fungi which he designates as "Aëthogametie" do not involve sex differentiation. Buller (*6*), Newton (*29, 30*), and Mounce (*27, 28*) interpreted it in terms of sexuality and designated such a fungus as "quadrisexual." They used the genetic notation introduced for such situations in fungi by Kniep (*19, 20, 21*).

Kniep (*21*) postulated that copulation of haplonts in Hymenomycetes is determined by genes which may occur as multiple allelomorphs. Thus, in such results as those recorded in Table 2, one is dealing with a haplodioecious fungus with two pairs of such allelomorphs localized in four different chromosomes. This type of fungus is designated as of the "tetrapolar" or "dihybrid" type. At first Kniep designated these genes as "sex factors" (*19, 20, 21*). Later, in harmony with the suggestion of Prell (*34*), Brunswik (*2*), Oehlkers (*31*), and others, he (*22*) dropped this term and, following the suggestion of Oehlkers, designated them as copulation factors. Consequently he replaced the phrase "pluripolar sexuality" by "pluripolarity of copulation-determining factors." He made the fundamental assumption that the presence of identical copulation factors prevents copulation. Thus *AB* can copulate only with *ab*.

Brunswik (*2*) has interpreted identical phenomena in his experimental material by assuming the operation of sterility factors. He assumed that autogamy is the fundamental process in both monoecious (homothallic) and dioecious (heterothallic) fungi; that is, both have the same genotypic constitution so far as sex is concerned. Heterothallism is determined by the addition of inhibiting or sterility factors, and mutability and allelomorphism are linked with these rather than with real sex factors. Specifically, in the case in hand, if one follows this conception, copulation of the haplonts is determined by the presence or absence of two sterility factors. Oehlkers (*31*) points out that this interpretation is more in line with Correns's theory of sex than Kniep's original conception. He is inclined, however, to use the phrase "copulation factors," but is opposed to the genetic formulations advanced by Kniep.[4]

The position of Vandendries (*45*) is not quite clear. In one instance, in contrasting Brunswik's "sterility factors" and Kniep's use of the concept "fertilizing factors," he states that in the critique of his experiments he has always adopted by pure opportunism Kniep's interpretation. In the same paper he states that both these theories lie in the realm of pure hypothesis; that with respect to the law of fertility between geographic races the theory of Kniep must admit an indeterminite number of determiners, whereas the theory of Brunswik permits consideration of the loss of a single determiner as a cause of exceptionally increased fertility; and that he would be inclined to adopt the point of view of Brunswik.

[4] This statement is based on the review by Kniep (*24*) of Vandendries's paper (*44*), which the present writer has not seen.

Further understanding of the fundamental causes operative in the production of more than two types of haplonts is dependent upon more detailed study of the phenomenon than was attempted in the work presented here. For this reason, the interpretation of results in this paper is based upon conceptions and conclusions—although not accepted in toto—that other workers have derived from more extensive investigations.

It has been assumed that the four types of haplonts are the result of segregation of factors carried by the fusion nucleus of the diplont. The fusion nuclei are said to contain two allelomorphic pairs of genes that determine the sexual nature of the individual haplonts. The four genes are thought to be borne separately on four chromosomes. Thus the genotypic formula for the fusion nucleus is written *AaBb*. It is seen that four genotypically different types of nuclei may follow chromosome segregation. The four possible genotypes would be *Ab*, *aB*, *AB*, and *ab*. Kniep postulated that crosses occur only between haplonts that have no duplication of genes. Thus the genotype *Ab* would be compatible with *aB* but not with *AB* or *ab*. The symbols used in Table 2 indicate genotypic differences in the four types of haplonts used in the present pairings. Thus it is seen that the haplonts designated as *ab* produced clamps when paired with all the haplonts under the symbols *AB*, but not with any of the other three types. Further analysis of the table shows that each group of haplonts behaved in a similar manner; that is, compatibility was found only between haplonts designated by opposite genetic formula.

Experiments by several investigators (*2*, *17*, *19*, *20*, *27*, *28*, *39*) have demonstrated that four genotypes are consistently present in the sporocarps, or hymenia, of 15 species of Hymenomycetes. Studies of the spores from individual basidia showed that some basidia bear two types of spores (*21*) and that other basidia bear four types of spores (*16*, *30*). This behavior is attributed to variation in the time of the reduction division. Inasmuch as the basidia are four-spored, two divisions must follow the fusion of the dicaryon in the basidium. If the first of the divisions is the reduction division, only two genotypes are possible for each basidium, and two basidia are necessary to produce the four genotypes in one fruiting body. The nuclei resulting from the second division would be genotypically identical with their sister nuclei. If, however, the reduction division occurred in the second division, four genotypes could be borne on the same basidium, as the two daughter nuclei of the fusion nucleus would each contain the four genes *AaBb*. Therefore, if reduction division in one of the daughter nuclei gave rise to genotypes having the formulas *AB* and *ab*, and the other nucleus produced genotypes represented by the formulas *Ab* and *aB*, spores of four genotypes would be produced on one basidium. In the present work no effort was made to obtain spores from individual basidia. The spores, as stated, were transferred from an arachnoid hymenium.

INTERCROSSING OF FALSE-ANTHRACNOSE CULTURES

Table 3 gives the results of a checkerboard cross of six false-anthracnose cultures, all obtained from apples grown in Washington, and a seventh culture (No. 279–A) isolated from a New York Baldwin. Laboratory studies had shown no difference in morphology or path-

ogenicity between the western strains and this eastern culture. The numbers used are the stock numbers under which the cultures have been carried in the laboratory. The technic employed was the same as that described for the preceding experiments. Examination of the cultures was made when the hyphae of the two strains had mingled thoroughly. As shown by Table 3, clamps were found in but one pairing, that between culture No. 266–A, isolated from Rome apples shipped from Benton City, Wash., and sent to the Chicago office from Detroit, and culture No. 274, isolated from Winesap apples shipped from Wenatchee, Wash., and inspected on the Chicago market.

TABLE 3.—*Results of all possible pairings of seven so-called false-anthracnose strains*

[− indicates absence of clamps; + indicates presence of clamps]

Culture	No. 145	No. 226	No. 248A	No. 266A	No. 273	No. 274	No. 279A
No. 145	−	−	−	−	−	−	−
No. 226	−	−	−	−	−	−	−
No. 248–A	−	−	−	−	−	−	−
No. 266–A	−	−	−	−	−	+	−
No. 273	−	−	−	−	−	−	−
No. 274	−	−	−	+	−	−	−
No. 279–A	−	−	−	−	−	−	−

The nodose septate form resulting from the cross fruited and was studied in the laboratory. It agreed in all morphological details, including size and form of hyphae, basidia, and spores (fig. 1, G), with both eastern and western perfect forms isolated from apples showing fisheye rot. Checks were made as usual, and examination showed all checks to be free from clamps.

The cross between Nos. 266–A and 274 was repeated five times. Each time clamps were produced by the mating, and check cultures remained negative. Duplication of the balance of the checkerboard gave identical results a second and a third time. None of the other cultures produced clamps when paired. Their failure to cross will be discussed later.

CROSSING OF FALSE ANTHRACNOSE WITH FISHEYE CULTURES

These 6 western cultures of false anthracnose were representative of about 50 sterile strains isolated from western apples during the last three years. The other strains died in the laboratory. The character of the lesions from which they were isolated, as well as the morphology and consistent sterility of the mycelium, are evidence that the causal organism of false anthracnose and these sterile strains are the same fungus. Although about 20 isolations had been made from lesions on apples grown in New York identical with these western lesions, culture No. 279–A was the only strain that had remained alive during the entire period of these studies. This lack of vitality under laboratory conditions, together with the identity in morphology and pathogenicity, indicates that the eastern and western sterile strains are the same organism. The fact that No. 279–A was isolated from an apple from a second lesion of which was isolated the perfect form producing fisheye rot, together with the production by crossing of two western strains of a perfect fungus identical morphologically and pathogen-

ically with the fisheye-rot organism, would seem conclusive evidence that the sterile strains producing false anthracnose and the sterile strains of the fisheye-rot organism are identical. Inoculation studies with the four strains of the fisheye-rot organism proved that these strains are equally pathogenic. Lesions produced by the perfect strain and by the four sterile strains produced by germination of its single spores are indistinguishable. These lesions are also indistinguishable from those produced by inoculation with each of the sterile strains from false-anthracnose lesions.

In order to check further the identity of these sterile strains, pairings were made with each of these seven strains and four single-spore strains from culture No. 211–C—Nos. 1, 2, 14, and 17. (Table 2.) The results are shown in Table 4.

TABLE 4.—*Results of all possible pairings of the so-called false-anthracnose strains with four genotypic strains of culture No. 211–C*

[− indicates absence of clamps; + indicates presence of clamps]

Culture	No. 1	No. 2	No. 14	No. 17	No. 266–A	No. 274	No. 145	No. 226	No. 248–A	No. 273	No. 279–A
No. 1	−	−	−	+	+	+	−	−	−	−	−
No. 2	−	−	+	−	+	+	−	−	−	−	−
No. 14	−	+	−	−	+	+	−	−	−	−	−
No. 17	+	−	−	−	+	+	−	−	−	−	−
No. 266–A	+	+	+	+	−	+	−	−	−	−	−
No. 274	+	+	+	+	+	−	−	−	−	−	−
No. 145	−	−	−	−	−	−	−	−	−	−	−
No. 226	−	−	−	−	−	−	−	−	−	−	−
No. 248–A	−	−	−	−	−	−	−	−	−	−	−
No. 273	−	−	−	−	−	−	−	−	−	−	−
No. 279–A	−	−	−	−	−	−	−	−	−	−	−

Because of the significance of these pairings they were made many times. It is seen that the single-spore cultures (Nos. 1, 2, 14, 17) maintained their previously noted behavior toward one another. Moreover, since previous pairings between the two western strains (Nos. 266–A and 274) had resulted in clamp formation as well as fruiting, positive results from the pairing of these two strains, obtained in this series, may be regarded as duplications of former findings.

Table 4 shows also that four of the western strains and the eastern strain did not form clamps with any of the strains with which they were paired. Although these five sterile strains have been paired in all possible combinations with each other and with the four types of haplonts from single-spore cultures six times, no clamp connections have ever been found in examinations of the pairings. This consistent maintenance of the haplophase necessitates the consideration of three possibilities. The first is the phylogenetic relationship involved. If the five sterile strains were too distantly related to the others with which they were incompatible, that is, different species, the negative results obtained would be expected. The negative results from interpairing the five variants would then indicate either that they were too distantly related to one another or that they were genotypically alike. However, cultural studies in the laboratory have disclosed no morphological, physiological, or pathogenic differences among these five sterile strains and any other haplonts studied. It is believed that the similarities noted render untenable

the hypothesis that a too distant relationship is responsible for the negative results.

The second possibility is that all the haplonts found belong to the same species, but that the affinities for these five sterile strains, although present in the progeny of the New York strain, have not been found. However, the clear-cut dihybrid behavior of the 30 single-spore cultures used in the previous work is rather strong evidence that only four genotypes are formed by this strain. Additional evidence that there are but four genotypes is provided by the following fact: No deviation from the dihybrid behavior was found when 30 single-spore cultures made from the F_1 generation were paired with each of the four genotypes that had been segregated from the parent generation. Each of the 30 haplonts formed clamps with some one of the original four genotypes. This typical dihybrid behavior among 60 haplonts seems sufficient evidence that representatives of all genotypes formed by this strain have been found.

The third possibility is occasional incompatibility, such as has been found to exist in other Hymenomycetes, among haplonts derived from individuals of the same species that were separated geographically. Vandendries (*45*) made studies of individuals of *Coprinus micaceus* from Luxemburg, eastern and western Canada, the Netherlands, Germany, and other widely separated localities. The behavior of the haplonts from these individuals was very erratic. Kniep (*23*) in reviewing this work stated that it furnishes proof that haplonts of the same species of different geographical origin can be sterile toward one another on an extensive scale. The erratic behavior among haplonts of individuals from different localities has been found by other workers in many species of Hymenomycetes and is attributed either to gene changes or to environmental factors that modify the development of the inherent genotypic tendencies. In the present work it is felt that the consistent incompatibility of the five haplonts discussed may be considered as an additional instance of such a phenomenon. Therefore, they are here regarded as haplonts of the same species even though they do not form clamps with any of the haplonts so far found in the species. This phenomenon will be more fully considered in the discussions that follow.

A further study of Table 4 shows that two of the strains (Nos. 266–A and 274) from western apples mated with one another and that each formed clamps with each of the four single-spore cultures (Nos. 1, 2, 14, 17), which represent the four types of haplonts from the New York culture No. 211–C. These results can not be interpreted in the light of the purely qualitative genetic conceptions thus far used in this paper. The fundamental premises previously used in this discussion are that duplication of the genes involved renders haplonts incompatible and that genotypic opposites are compatible. The theoretical genetic formula of the fusion nucleus previously presented, *AaBb*, permits but four genotypes. The assigning of the genetic formulas, which follow, is based on previous crossings of the haplonts. (Tables 2 to 4.) The assignment of the notation *aB* to strain No. 266–A and of *Ab* to strain No. 274 indicates that they are genetic opposites and should mate. The finding of clamps in each of the six pairings that have been made between these two is seen to be in accord with the notation. The qualitative genetic notation is

supported also by the behavior of cultures Nos. 1, 17, 14, and 2, which have, respectively, the genetic notations *AB*, *ab*, *Ab*, and *aB* when crossed among themselves (Tables 2 and 4), in that the presence or absence of clamps has been consistent with the genotypic symbols assigned to the paired haplonts.

In additional studies cultures Nos. 274 and 266–A were each paired with each of the above-mentioned single-spore cultures in two Petri dishes on string-bean agar. Microscopic study showed that clamp formation had again occurred in each of the eight pairings. Following these qualitative results a quantitative study was made. An extensive series of mounts of the mycelia was made from each of the pairings in the two plates and studied microscopically. A sufficient number of systematic studies was made to permit the mapping of the various conditions of the different areas within the traced outlines of the colonies. The results are shown in Figure 2.

An analysis of the diagrams shows that not only were the results of mating qualitative, as indicated by the presence or absence of clamps, but quantitative as indicated by the number and completeness of clamps present in each pairing. Ordinarily when two haplonts mate, normal clamps occur and oidia are absent at the point of contact of the two haploid mycelia, and the condition is reversed in both haplonts a short distance from the point of contact. In pairings held in the laboratory for some weeks it has been found that, as time passed, a decrease in haploid hyphae containing septa and producing oidia was accompanied by an increase in diploid hyphae that formed clamps and did not produce oidia. In an examination of positive pairings 3 to 4 weeks old it was found that ordinary septa were very rare and that clamps were present in abundance, while oidia were no longer found. These findings indicate very strongly that when haplonts copulate a progressive decrease of the haplophase is accompanied by a progressive increase of the diplophase. In the present case, however, no such consistent regularity of progression was found, but degrees of completeness of copulation were noted, the quantitative conception of copulation being based on differences in the included pairings and in the different areas in each pairing. Such differences involved the presence of oidia and ordinary septa (fig. 1, A), which were accepted as evidence of haploid hyphae, and the relative normality and number of clamp connections, which were considered proof of diploid hyphae. Clamps that were incomplete or variants (fig. 1, B) were considered to indicate a lesser degree of compatibility than complete normal clamps (fig. 1, C). The accompanying genetical interpretation (Table 5) of some of the crosses shown in Table 4 is included here to facilitate the discussion of these quantitative phenomena.

The genetic formulas used are those already assigned to the different strains. The plus and minus signs, as in other tables, indicate the presence or absence of clamp connections. The pairings are numbered consecutively for more ready reference.

An examination of Table 5 shows that clamps were formed in each instance in which the individual contributions of the two haplonts resulted in the production of the genetic formula *AaBb* that has been considered normal for the fusion nucleus. This is seen in pairings Nos. 2, 5, 7, 10, 18, 20, 23, 25, 28, and 33. Each of these crosses has been strong; that is, it has produced many normal clamps. Nos.

2 and 7 are duplicate crosses of Nos. 266–A and 274, which have always given strong results. Three pairings that were held in the laboratory fruited copiously. Nos. 18 and 33 are duplicate pairings of Nos. 1 and 17 and Nos. 23 and 28 are duplicates of the cross between Nos. 2 and 14, which have repeatedly given strong, positive results with copious clamp formation and sporulation.

TABLE 5.—*Genetic notation of the pairings of two western and four eastern haplonts*

Haplonts		No. 266–A			No. 274			No. 1		
No.	Genetic notation	Pairing No.	Clamps present (+) or absent (−)	Genetic notation	Pairing No.	Clamps present (+) or absent (−)	Genetic notation	Pairing No.	Clamps present (+) or absent (−)	Genetic notation
266–A	*aB*	1	−	*aBaB*	2	+	*AbaB*	3	+	*ABaB*
274	*Ab*	7	+	*aBAb*	8	−	*AbAb*	9	+	*ABAb*
1	*AB*	13	+	*aBAB*	14	+	*AbAB*	15	−	*ABAB*
2	*aB*	19	+	*aBaB*	20	+	*AbaB*	21	−	*ABaA*
14	*Ab*	25	+	*aBAb*	26	+	*AbAb*	27	−	*ABAb*
17	*ab*	31	+	*aBab*	32	+	*Abab*	33	+	*ABab*
		No. 2			No. 14			No. 17		
266–A	*aB*	4	+	*aBaB*	5	+	*AbaB*	6	+	*abaB*
274	*Ab*	10	+	*aBAb*	11	+	*AbAb*	12	+	*abAb*
1	*AB*	16	−	*aBAB*	17	−	*AbAB*	18	+	*abAB*
2	*aB*	22	−	*aBaB*	23	+	*AbaB*	24	−	*abaB*
14	*Ab*	28	+	*aBAb*	29	−	*AbAb*	30	−	*abAb*
17	*ab*	34	−	*aBab*	35	−	*Abab*	36	−	*abab*

The remaining pairings that resulted in clamp formation, Nos. 3, 4, 6, 9, 11, 12, 13, 14, 19, 26, 31, and 32 of Table 5, really represent but six different crosses because of the duplication in the checkerboard. Examination of the table shows that although clamps were formed in each instance, the theoretical formulas for the resulting fusion nuclei deviate from the assumed *AaBb* normal. It is also seen that degrees of deviation occur.

For convenience the numerical designation of the crosses in the genetic Table 5 is duplicated in the diagrammatic sketch of the Petri dishes. (Fig. 2.) Comparison of the figures in relation to the crosses thus far discussed discloses a quantitative correlation. Thus cross No. 5 is the strongest experimentally (fig. 2, A) and is the only one of the four crosses between 266–A and the four genotypes that has a normal genetic formula. Cross No. 4 in Table 5 is seen to show complete duplication in both pairs of allelomorphs, and reference to the diagram (fig. 2, A) shows it to be the weakest cross. Crosses Nos. 3 and 6 each show duplication in one of the included allelomorphic pairs and are both seen to be approximately midway in strength of crossing between the other two. The slight difference between crosses Nos. 3 and 6, shown in the figure, might be due to the relative importance of the genes involved or to the relative vigor of the two genotypes. Thus it is seen that the quantitative aspects of the theoretical genetic conceptions are supported by the experimental determinations.

It is believed that the sequence of the experimental findings and the theoretical applications are worthy of note. The microscopic

studies and mapping were completed before the genetic interpretations were attempted. An examination of the second diagram (fig. 2, B) reveals a complementary situation in the crosses between strain No. 274 and strains Nos. 1, 2, 14, and 17. As stated previously, strain No. 274 has repeatedly proved to be the genotypic opposite of strain No. 266–A. Therefore, opposite behavior would be expected. Comparison of crosses Nos. 10, 11, 9, and 12 in the diagram shows that the expectations are fulfilled. It is seen that in these four crosses the strongest one occurs between strain No. 274 and strain No. 2 (cross No. 10), whereas strain No. 2 (fig. 2, A) formed the weakest cross with strain No. 266–A (cross No. 4). Inasmuch as strains Nos. 2 and 14 are affinities and strains Nos. 266–A and 274 are also genotypic opposites, this behavior is in accord with expectations. Further analysis of the diagram shows that strains Nos. 1 and 17 occupy a midway position in strength of crossing with strain No. 274 (crosses Nos. 9 and 12). This is consistent with their behavior with strain No. 266–A (crosses Nos. 3 and 6). (Fig. 2, A.) To complete the consideration of the complementary relationship between strains Nos. 266–A and 274, attention is directed to cross No. 11 between strain No. 274 and strain No. 14. It is seen that the pairing of No. 14 with No. 274 resulted in the weakest cross.

A consideration confined to the relationships between strains Nos. 274 and 2 and between Nos. 266–A and 14 is in accord with a purely qualitative genetic interpretation involving two pairs of allelomorphs. The crossing of strains Nos. 266–A and 274 with strains Nos. 1 and 17, and of No. 266–A with No. 2, and of No. 274 with No. 14, can not be explained by this hypothesis. Reference to Table 5 shows that identical genetic formulas have been assigned to both positive and negative pairings, as in crosses Nos. 13 and 16. Cross No. 16 shows agreement in but one pair of genes and is negative, whereas cross No. 11 shows agreement in both pairs of genes and is positive.

DISCUSSION OF CROSSINGS

In the present work no purely genetic studies, such as interpairing or back crossing of hybrids, have been made. The results of such studies with other Hymenomycetes by Kniep (*22*, *23*) support the contention that gene changes are involved. In view of this fact, the first question that presents itself is the correctness of the distinction between positive and negative pairings. The presence or absence of clamps is, as has been said, the accepted criterion. In the work presented in this paper efforts have been made to confirm diagnoses based on the presence or absence of clamps by subsequent presence or absence of sporulation in the cultures. Fruiting has occurred with some strains in seven to nine days on string-bean agar. However, as has been stated, the fruiting of the fungus is erratic. The writer has not been able to induce sporulation at will with any of the nodose septate strains studied. This has been true of all perfect forms isolated from apple as well as of the perfect forms that resulted from crosses in the laboratory. Therefore, although sporulation when found may be considered as positive evidence, the absence of fruiting can not be accepted as negative evidence. In the intercrossing of the six haplonts here considered (Nos. 266–A, 274, 1, 2, 14, and 17)

spores have been found, as stated in different places herein, in the crosses between Nos. 266–A and 274, Nos. 1 and 17, and Nos. 2 and 14, but not as yet in any of the other crosses.

The formation of clamps that has occurred in the pairings between the eastern and western haplonts and the agreement in morphology and pathogenicity seem ample proof that they belong to one and the same species. Hanna (*17*), working with geographical strains of *Coprinus lagopus* from England and from Canada, found that haplonts from the two geographical strains reacted positively. She stated:

> The fact that hyphal fusions took place between the English and Canadian strains, resulting in the formation of clamp-connexions, furnishes a conclusive proof that the Coprinus lagopus found in England is identical with the species occurring in Canada.

Vandendries (*41*) reported that he had crossed *Panaeolus campanulatus* and *P. fimicolo*, but both Kniep (*22*) and Brunswik (*3*) state that all efforts to cross species have failed and that this cross had not been repeated and is questionable.

The complete fertility recorded in Table 5 between each of the two western haplonts and the four genotypes from the eastern strains is in accord with the findings of other workers. For example, Hanna (*17*) found that the individual fruit bodies of *Coprinus lagopus* consistently produced four genotypes. That is, the testing of the haplonts resulted in typical dihybrid results such as are recorded in Table 2 in this paper for *Corticium centrifugum*. However, when she paired any haplont from a fruit body of one locality with the four genotypes from a fruit body from another locality, each pairing was positive. In 694 pairings that were made between haplonts from different fruit bodies she found that while each fruit body produced but 4 "sexes" the 6 fruit bodies in the aggregate produced 24 "sexes." She stated:

> Further investigations may show that these sexual strains are to be found generally throughout the heterothallic Basidiomycetes, and that each species is made up of a definite number of such strains.

Other instances of haplonts showing a typical monohybrid or dihybrid type of behavior when paired with other haplonts from the same fruiting body but copulating with all the haplonts from different fruiting bodies of the same species, obtained from slightly or widely separated localities, have been reported by Kniep (*21*, *22*, *23*) for *Schizophyllum commune*, *Aleurodiscus polygonius*, *Collybia velutipes*, *C. conigena*, *Armillaria mucida*, and *Coprinus fimetarius;* by Brunswik (*2*, *3*, *4*) for *C. fimetarius*, *C. comatus*, *C. niveus*, *C. picaceus*, *C. lagopus*, and *C. friesii;* by Vandendries for *Panaeolus campanulatus* (*41*) and for *C. radians* (*42*); and by Newton (*29*) *for C. rostrupianus*.

Taking into consideration the results of these workers, it would seem that the two western haplonts studied in this paper represent two of four genotypes and that the other two genotypes have not yet been found. A brief résumé and correlation of the presentation in this paper with the findings of other workers follows.

The phenomena reported here for *Corticium centrifugum*, that is, the dihybrid behavior recorded in Table 2, the interincompatibility and intraincompatibility of five haplonts, as shown in Tables 3 and 4, and the apparent quantitative manifestation illustrated in the diagrams (fig. 2), are of the same order as those that have been reported

for other Hymenomycetes. A comprehensive discussion of the subject will be found in papers by Kniep (*23*) and Brunswik (*5*).

Many of the Hymenomycetes, which are dioecious (heterothallic), have been shown, by crossing of the haplonts from individual fruiting bodies, to follow the clear-cut dihybrid ("tetrapolar" or "vierer-schema") type of behavior recorded for *Corticium centrifugum* in Table 2. A smaller number have shown a monohybrid ("bipolar" or "zweierschema") type of behavior, of which there is no indication in *C. centrifugum*.

In addition to the previously discussed deviation from the "tetra polar" condition that occurs when haplonts from different fruiting bodies are paired, deviations have been found to occur in the relationship of haplonts from the same fruiting body. Reports of deviations from either the "bipolar" or "tetrapolar" type of behavior in the haplonts from a single fruiting body have been made by Vandendries (*41, 44, 45*) for *Panaeolus campanulatus, P. separatus,* and *Coprinus micaceus;* by Kniep (*22*) for *Schizophyllum commune;* and by Brunswik (*4*) for *C. picaceus*. However, enough consistent regularity was found to indicate that merely a deviation from the type is involved rather than an entirely different phenomenon. Brunswik (*4*) reported for *C. picaceus* a 25 per cent deviation from the typical dihybrid behavior and termed the phenomenon "Durchbreckungskopulation."

All the haplonts of *Corticium centrifugum*, when carried in the laboratory in pure culture, have maintained their sterile condition. Some of the strains have been in culture for nearly three years, but no clamp formation or fruiting has been observed. Other workers have found a spontaneous changing from the haplophase to the diplophase. Vandendries (*42*) terms this phenomenon "l'hétéro-homothallisme." This phenomenon has been reported by Kniep (*20*) for *Schizophyllum commune* and *Collybia velutipes;* by Vandendries (*42*) for *Coprinus radians* and *C. micaceus;* by Hanna (*17*) for *C. lagopus;* and by Newton (*29*) for *C. rostrupianus*.

It is seen that investigations of dioecious Hymenomycetes thus far have indicated that either a monohybrid or dihybrid behavior is consistent enough to be considered typical or normal. It is also clear that deviations of different kinds occur. Kniep (*22, 23*), in maintaining his fundamental assumption that presence of like genes in any two haplonts prevents copulation and that genotypic opposites do copulate, assumed that gene changes cause these deviations. He contended that no completely different genes are involved, but that the genes are localized in homologous chromosomes in the same position, but are slightly changed quantitatively. In other words, the phenomenon of multiple allelomorphs is involved. Thus, the genetic formula of the fusion nucleus may change from *AaBb* to $A'a'B'b'$, or the change may affect but one pair of allelomorphs. The haplonts containing the modified genes maintain the dihybrid ratio between each other, but each will copulate with any haplont containing unmodified genes. The disruption of the typical monohybrid or dihybrid ratios among haplonts from the same fruiting body, previously referred to, and the complete interfertility of haplonts from different localities are attributed to quantitative gene changes.

Cases of complete intersterility are also reported. Kniep (*23*) has reported that a haplont of *Schizophyllum commune* did not copulate with any of the haplonts from another fruiting body. Vandendries

(*42*) has reported a similar phenomenon for *Coprinus radians*. Brunswik (*4*) found the same situation in *C. comatus*. The most striking example is that reported by Vandendries (*45*) for *C. micaceus*. Interpairing of haplonts from different geographical strains showed two types of deviations. Some of the haplonts were incompatible, while others showed increased compatibility. It is believed that the incompatibility in the case of the five haplonts discussed in this paper and the complete compatibility of the other two western haplonts comprise an analogous situation. Kniep (*23*) reported that the haplonts of two fruiting bodies of *Collybia cirrhata* collected in one locality were completely interfertile, but that haplonts from two other fruiting bodies collected in different places in another locality were absolutely intersterile. The completely interfertile forms are easily explained by the previously advanced theory of quantitatively altered genes. However, the complete intersterility of the two other fruiting bodies can not be explained by the complementary assumption of identity of genes in all eight component haplonts, for since the four haplonts of each fruiting body follow a pure or slightly modified dihybrid formula they must be different genotypically. Kniep considers that the intersterility may be due to too great a quantitative difference in the genes or to secondary factors.

The spontaneous change of mycelia from the haplophase to the diplophase is also assumed by Kniep (*23*) to be the result of gene changes. In a monohybrid type the change involves one allelomorphic pair while in a dihybrid two pairs of genes are modified.

The genetic interpretation of the behavior of the Hymenomycetes advocated by Kniep (*21*, *22*, *23*) thus embraces the conception that the forms are highly mutable. This interpretation finds support in extensive purely genetic studies by Kniep and other workers. Kniep (*23*), however, states that he has at present no satisfactory explanation for all the deviations that have occurred in his tests of *Schizophyllum commune*, and in those of Vandendries (*45*) with *Coprinus micaceus*, and in those of Brunswik (*2*) with *C. fimetarius*. Possibly they may necessitate an entirely different interpretation. At present the genotypic explanation serves better than any other as a working hypothesis.

In the light of this brief consideration of the literature, it is seen that the results of the study of the apple pathogene presented in this paper are in accord with the findings of other workers for numerous Hymenomycetes. No deviations have been found in the studies of the apple organism that have not been reported as occurring within a single species of other Hymenomycetes.

TAXONOMY

Since the fungus described in this paper compares so closely in pathogenicity and morphology with the one described by Eustace as *Hypochnus* sp., there is little doubt that it is the same fungus. Taxonomic studies carried out in the present work have shown that, although much attention has been given to the group, a great deal of confusion still exists in regard to the taxonomy of Hypochnus and closely related genera.

The genus Hypochnus was described by Fries (*14*) in 1818. In 1874 (*15*), however, he placed Hypochnus as a subgenus under Cor-

ticium, reserving for Hypochnus those species of Corticium which possessed a floccose or tomentose to almost powdery hymenium. In 1881 the genus Hypochnus was amended by Kärsten (*18*), who included in it most of the congeneric forms originally included by Fries, but excluded such as fell logically into the genus Corticium. Hypochnus as amended by Kärsten includes the synonymous genus Tomentella ascribed to Persoon (*33, v. 2, p. 18–19*) but first published as a genus by Patouillard (*32*) in 1887. The genus Hypochnus as given by Saccardo (*35*) is a composite group based on the old Fries classification but includes forms which Fries had later discarded from the genus. Many species described by Saccardo under Hypochnus are now placed in Corticium, Peniophora, and related genera. Engler and Prantl (*12*) and Stevens (*37*) separate the genus Hypochnus Ehrenberg (*11*) from closely related genera on the basis of spore color and number of sterigmata, and following the suggestion of Schroeter (*36*), place it in the family Hypochnaceae, which is separated from the Thelephoraceae on the basis of the absence in the former group of a compact layer of closely aggregated basidia forming a true hymenium. H. and P. Sydow (*38*) advise the dropping of Hypochnus, in the sense of Schroeter, and the assignment of the species to their proper places in other genera. Burt (*7*) agrees that Hypochnus, in the sense of Schroeter, must be abandoned, and follows Kärsten in placing under Hypochnus only resupinate species with colored echinulate spores. Resupinate forms with smooth hyaline spores, placed by Engler in Hypochnus, would, therefore, be excluded. Burt (*8*) assigns to the genus Corticium those species, both hypochnoid and compact, which are always resupinate, have colorless spores, and lack cystidia, excepting a few parasitic forms placed in Exobasidium.

The fungus under discussion fits closely Burt's description of *Corticium centrifugum* (Lev.) Bres., which is synonymous with the *Hypochnus centrifugus* Lev. described by Saccardo (*35, v. 6, p. 654*). Burt (*8*) stated, however, that *C. centrifugum* is "only rarely nodose septate." Lindau and Ulbrich (*26*) in their description of *C. centrifugum* say that clamp connections are abundantly present. It is highly probable that the heterothallic nature of the fungus is responsible for these contradictory statements. As clamp connections are not formed in the sterile strains and are consistently present in the perfect strains, some variation is to be expected. In material in which fusion had recently occurred clamp connections would be present in some hyphae and not in others. (Fig. 1, D, and Fig. 2.) Therefore, the number of clamp connections found in material collected in the field would be proportional to the relative proportion of sterile and perfect mycelium present in the specimen. Examinations of crosses made with compatible strains of this fungus in the laboratory have shown great variations in the number of clamp connections present. Soon after crossing takes place clamps occur only near the point of contact, while the rest of the colony growth is made up of hyphae with ordinary septa and oidia. (Fig. 2.) Later the new growth at the periphery of the colonies shows perfect clamps (fig. 1, C; fig. 2, A, 14, and B, 2), and in the case of strong crosses some imperfect or incomplete clamps (fig. 1, B) even develop in the old growth near and over the inoculum (fig. 2, A, 14, and B, 2.) When the culture is about 2 weeks old clamps are the rule and ordinary septa the exception.

Corticium centrifugum is rather widespread in the United States and has been reported from three of the States (New York, Washington, and Oregon) from which the apple-rotting fungus has been collected. This fact further substantiates the relegation of the apple fungus to this species.

DESCRIPTION OF CORTICIUM CENTRIFUGUM FROM PURE-CULTURE STUDY

Mycelium spreading over the substrate and forming a delicate white mat, varying from closely appressed to fluffy and occasionally zoned; center of mat thicker than extremities; older cultures on bean pods or apple twigs forming hyphal knots, or plectenchymic cushions 3 to 4 mm. in diameter, white when young, brownish (cinnamon buff to avellaneous)[5] when old; hyphae smooth, white, loosely interwoven, not incrusted, diameter variable, averaging 3.5 μ, dihybrid; clamp connections characteristically present in perfect forms (fig. 1, C, E), absent in sterile strains (fig. 1, A); hymenium arachnoid, powdery, indefinite; basidia borne directly on hyphae (fig. 1, F, G), clavate 3.5 to 5.5 by 9 to 20 μ, bearing four sterigmata from 1.85 to 5.5 μ (fig. 1, H); no cystidia; spores subglobose to ovate, smooth, hyaline, flattened on one side, 3.4 to 3.6 by 5.4 to 7 μ average, 2.7 by 3.7 μ minimum, 4.6 by 8.3 μ maximum (fig. 1, F, G, H). Oidia present in sterile strains. (Fig. 1, A.)

Habitat: Parasitic on apple fruit. Occurs in New York, Illinois, Washington, Idaho, Oregon, and Virginia.

SUMMARY

The diseases known as Hypochnus rot, fisheye rot, and false anthracnose are identical and are caused by the same fungus, which was previously designated as *Hypochnus* sp., but which is here identified as *Corticium centrifugum* (Lev.) Bres.

The decay in apples progresses at the ordinary commercial cold-storage temperature of 32° F. Indications are that infection occurs in the orchards and that the disease develops but does not spread in transit or storage.

The fungus has been found to grow well on artificial media. Vegetative growth is favored by high humidity and is extremely sensitive to desiccation.

Studies of the fungus have shown that it is heterothallic and that individual perfect strains produce four types of haplonts. The results of crossing studies made with sterile strains have been interpreted genetically and are in accord with the findings of other workers for other Hymenomycetes. The identity of eastern and western strains of the fungus based on morphological and pathogenic similarities has been supported by crossing of sterile strains.

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SOME CONDITIONS AFFECTING THE STORAGE OF PEPPERS [1]

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INTRODUCTION

A quantity of green peppers (Capsicum) grown in the vicinity of Washington, D. C., and stored in a local cold-storage house at a temperature of about 0° C. from October 24 to November 20, 1924, was inspected at the request of the owner. The fruits were in various stages of deterioration and were thought to have been injured by freezing. However, a careful examination of the lesions led to the belief that the trouble was caused by a fungus rather than by freezing, (1) because the lesions were more definitely localized than is usual in freezing injury, and (2) because a septate mycelium was found in affected areas. Fifty-one isolations from lesions in all stages of development were made from the diseased peppers to carrot-agar plates, with the following results: 46 plates yielded pure cultures of a species of Botrytis, 4 were sterile, and 1 yielded an unidentified fungus. Typical lesions developed in pepper fruits inoculated with the Botrytis.

A fruit rot of pepper caused by *Botrytis cinerea* Pers. which occurred at the Missouri Botanical Garden in 1911 was reported by Peltier [2] in 1912. The disease was characterized as follows:

> The peduncles were covered with a dark-brown mass of conidia and mycelium, which so weakened the tissues that the fruit soon fell to the ground. On opening the peppers a number of large, flat, crustlike sclerotia, 1 cm. long and 0.5 cm. wide, were found to fill the interior. In most cases the seeds were also covered with the crustlike masses.

This appears to be the only report on record of a disease of pepper caused by *Botrytis cinerea*, the fungus responsible for the storage rot.

The purpose of this paper is (1) to describe the freezing injury of peppers and the Botrytis disease as it develops in storage; (2) to submit data regarding the cause of the disease; (3) to report the effect of certain storage temperatures and humidities on development of the disease; and (4) to present data, obtained in connection with some of the storage tests, showing the influence of temperature and humidity on shriveling, ripening, and infection with and development of anthracnose (*Colletotrichum nigrum* E. and H.).

[1] Received for publication Jan. 15, 1930; issued September, 1930.

[2] PELTIER, G. L. A CONSIDERATION OF THE PHYSIOLOGY AND LIFE HISTORY OF A PARASITIC BOTRYTIS ON PEPPER AND LETTUCE. Missouri Bot. Gard. Ann. Rpt. 23: 41–74, illus. 1912.

DESCRIPTION OF FREEZING INJURY

A number of freshly gathered green peppers were frozen under controlled laboratory conditions, and a study was made of the symptoms of injury and the temperature at which the peppers freeze. It was found that freezing injury usually is not difficult to recognize. This injury is characterized by a soft, watery, flabby texture of the affected tissues (pl. 1, C), which may be restricted to a limited area in case of slight exposure or which may include the entire pepper. This is in contrast to the firm, brittle tissue of an uninjured pepper. (Pl. 1, A.) The external color of a pepper injured by freezing is darker than that of an uninjured pepper and is characterized by a dead and water-soaked appearance. The stem is usually soft and darker in color than that of an unfrozen pepper, and the epidermis shows a tendency to slough off when handled. When a frozen pepper is cut across, water quickly collects over the cut surface of the affected portion of the rind and drips off very freely if the tissue is squeezed. In addition, these surfaces are dark and water-soaked without the traces of white usually noted in the rind, in the segment partitions (septs), and in the core of the sound fruit. In cases of severe injury the seeds are brown in color instead of light green to white.

Plate 1 shows cross sections of a frozen and an uninjured pepper. In Plate 1, D, can be seen the dark color of the core and surrounding seed and to some extent the general state of collapse due to freezing injury. The white core and light-colored seed and the generally turgid appearance of an uninjured pepper are shown in Plate 1, B.

The freezing point as determined by the thermoelectric method was found to range from $-1.15°$ to $-0.99°$, with an average of $-1.06°$ C.

DESCRIPTION OF BOTRYTIS ROT

Most of the Botrytis-rot lesions were circular to elliptical, although some were irregular in outline due to the coalescence of two or more lesions. They varied in size from a mere point to an area so large that it involved most of the fruit. The color of the smaller lesions resembled a vertiver green,[3] which usually persisted at the margin of the larger ones. The central areas of the larger lesions varied from Isabella color, ecru-olive, and light brownish olive to buffy olive. The color of a given lesion seemed to vary more or less in this central region, depending on the amount of light, the angle at which light struck it, and the variation in the reflected light from the glazed surface of the pepper. The epidermis in the necrotic areas remained intact and could be peeled off readily. The underlying tissue had lost its coherence and was soft and watery. Some of the lesions, especially the smaller ones, conformed to the contour of the fruit, while others were slightly sunken, with sharp margins. In many of the larger lesions the surface was wrinkled and the diseased tissue tended to collapse toward the center of the fruit. The gray mass of conidia and mycelium may sometimes be seen on the surface of the fruit when the epidermis is broken. An excellent representation of the lesions is given in Plate 1, E and F.

[3] RIDGWAY, R. COLOR STANDARDS AND COLOR NOMENCLATURE. Pls. XXX and XLVII. Washington, D. C. 1912.

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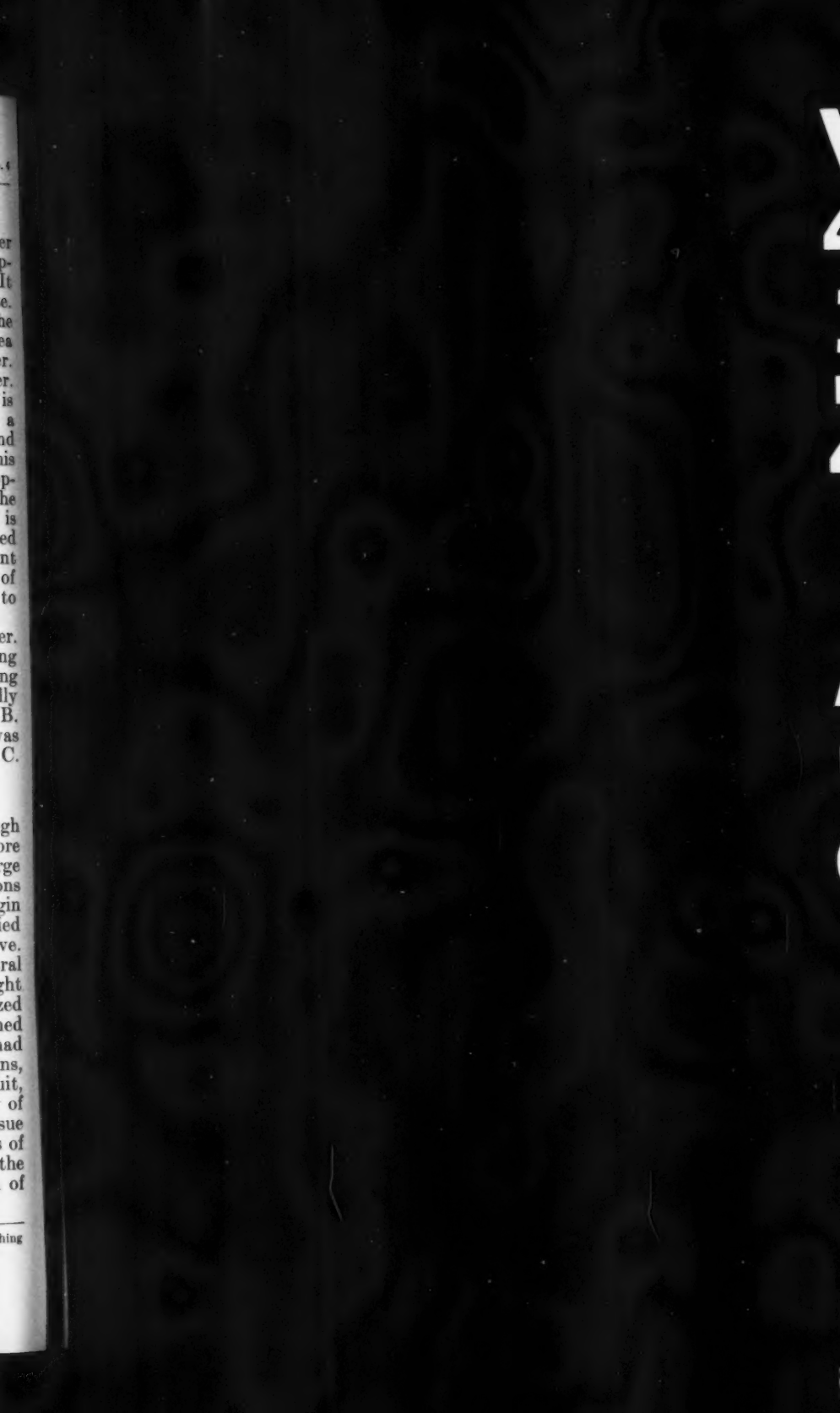

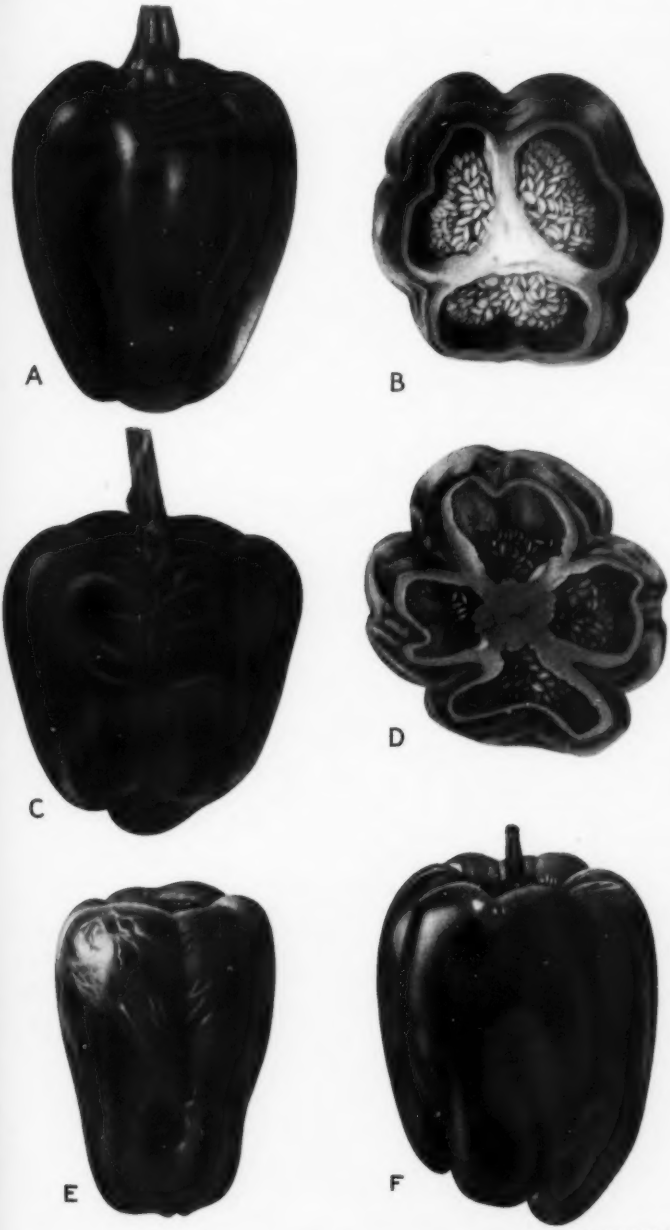

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A, Uninjured green pepper fruit; B, cross section of uninjured fruit; C, fruit injured by freezing; D, cross section of fruit injured by freezing; E, F, pepper fruits showing lesions of different sizes caused by *Botrytis cinerea*

(Colored drawings for A and C were made by Miss M. D. Arnold and for B, D, E, and F by R. C. Steadman.)

INOCULATION EXPERIMENTS

The results recorded in Table 1 were obtained from four experiments carried out to determine the pathogenicity of the Botrytis obtained from the foregoing isolations. In each experiment the peppers were washed with soap and water and rinsed in tap water. In experiment 1 an attempt was made to sterilize more completely the surface of the fruit by sponging with a wad of cotton saturated with 95 per cent ethyl alcohol. The peppers were inoculated by inserting a mixture of agar, mycelium, and spores from carrot-agar plates into the rind tissue by means of a sterile needle. The check fruits were merely punctured with a sterile needle. In experiments 1 and 2 a pure-line strain of Botrytis obtained from the original isolations was used, and in experiments 3 and 4 a reisolation from experiment 2 was employed. In each experiment both the inoculated fruits and the checks were stored under identical conditions in either moist chambers or 4-quart "till" baskets at the temperatures given in Table 1. Fifty-five infections, or 87 per cent, resulted from 63 inoculations. Only one infection occurred in the checks, and this did not occur at the point of puncture. Botrytis was obtained 24 times out of 26 isolations. The lesions were typical of those from which Botrytis was originally obtained.

TABLE 1.—*Results of inoculation experiments with Botrytis on green pepper fruits*

Experiment No.	Temperature of incubation	Inoculated fruits				Reisolations		Checks		
		Fruits used	Inoculations	Infections	Infection	Made	Yielding Botrytis	Fruits used	Needle punctures	Infections
	°C.	*Number*	*Number*	*Number*	*Per cent*	*Number*	*Number*	*Number*	*Number*	*Number*
1	22-25	3	7	5	71	[a] 8	8	3	5	0
2	15.5	11	11	11	100	10	8	8	8	0
3	15.5	30	30	25	83	------	------	5	5	[b] 1
4	10.0	15	15	14	93	8	8	5	5	0
Average	------	------	------	------	87	------	------	------	------	------
Total	------	59	63	55	------	26	24	21	23	1

[a] 2 isolations were made from each of 4 lesions in experiment 1. In the others each isolation was made from a separate lesion.
[b] This infection occurred at a point other than the puncture.

It is believed that these results, together with those discussed later, establish the fact that the necrotic condition of the peppers described was caused by this Botrytis.[4]

STORAGE EXPERIMENTS

DEVELOPMENT OF BOTRYTIS ROT IN DIFFERENT LOTS OF PEPPERS AT LOW TEMPERATURES

Five experiments were conducted to determine whether or not the storage of green pepper fruits at low temperature would normally result in infection by Botrytis in the absence of artificial inoculation. The fruits used in four of the experiments were purchased on the local

[4] H. H. Whetzel, of Cornell University, to whom the organism was submitted for identification, expressed the belief that it belonged to the *cinerea* type of Botrytis. The pathogene has been given the accession number 146.

market and were of unknown variety. The peppers employed in the fifth experiment were grown at the Arlington Experiment Farm, Rosslyn, Va., and were a mixture of the following varieties: Sweet-Meat Glory, Ruby King, Ruby Giant, and Bull Nose. Of the lots obtained on the market, one came from Florida, one was grown in the vicinity of Washington, D. C., and the origin of the other two is unknown.

Only sound fruits were used. They were stored in refrigerator rooms 8 feet wide, 14 feet long, and 11 feet high, in either 4-quart till baskets or 16-quart hampers. The results obtained are recorded in Table 2.

TABLE 2.—*Influence of low temperature on infection of pepper fruits by Botrytis*

Experiment No.	Where peppers were grown	Date of storage	Duration of storage	Temperature of storage	Fruits used	Infected	Lesions	Diameter of lesions	Isolations made	Times Botrytis was isolated	Other organisms isolated
			Days	*°C.*	*Number*	*Number*	*Number*	*Mm.*	*Number*	*Number*	
1	Not known (obtained on market, Washington, D. C.).	1924	57	−1	3	2	(a)				
		1924	57	0–2	6	6					
2	Vicinity of Washington, D. C.	1926	48	0–2	17	15	43	1–40	15	12	2 plates yielded Penicillium, and 1 an unidentified fungus.
3	Not known (obtained on market, Washington, D. C.).	1926–27	36	0–2	25	3	3		2	1	1 plate, sterile.
		1926–27	43	0–2	25	a 6	6		5	0	2 plates yielded Mucor sp., 1 Penicillium sp., and 2 bacteria.
4	Florida	1926–27	29	0–2	50	a 4	4		4	0	2 plates yielded Mucor sp., 1 a bacterium, and 1 was sterile.
5	Arlington Experiment Farm, Rosslyn, Va.	1927	41	0–2	54	54	(b)	1–50	24	19	5 plates, sterile.

a The lesions in these 2 cases were slightly atypical, being greener than the normal.
b Numerous.

The character of the lesions that developed was typical of Botrytis rot, the percentage of the fruits infected was high, and the number of lesions in experiments 1, 2, and 5 was large. Botrytis was obtained in most of the isolations made. The lesions that developed in experiments 3 and 4 were hardly typical of Botrytis rot, being slightly greener than normal lesions. The resemblance to the normal Botrytis lesions, however, was sufficiently marked to justify making isolations from them. Only a few fruits were infected, and the number of lesions was small. Botrytis was obtained only once out of 11 isolations. Four plates yielded a Mucor, three showed bacteria, one a Penicillium, and two were sterile.

The foregoing data justify the following conclusions: (1) Different lots of peppers do not react uniformly to Botrytis infection when stored at temperatures ranging from 0° to 2° C., and (2) peppers obtained on the market at Washington, D. C., or grown locally may become infected by Botrytis when stored at low temperatures for long periods.

INFLUENCE OF TEMPERATURE, HUMIDITY, AND TIME ON INFECTION OF PEPPERS BY BOTRYTIS

Two experiments were conducted during the fall of 1928 to determine the effect of certain temperatures and humidities on the infection of pepper fruits by Botrytis during storage. Ruby King was the variety employed in both cases. It was grown at the Arlington Experiment Farm and stored in galvanized-iron chambers 40 inches high, 42 inches long, and 35 inches wide, except in two cases (the two humidities at 13° C., Table 3), where they were stored in insulated refrigerated rooms 8 by 8 by 8 feet.

There were nine galvanized chambers, three of which were located in each of three insulated refrigerated rooms 8 feet wide, 14 feet long, and 11 feet high. The temperature was automatically controlled at 0° C. in one of these rooms, at 4.5° in another, and at 10° in the third. Three humidities were maintained at each temperature by means of water and calcium chloride in evaporating pans. The low, medium, and high humidities at one temperature were fairly comparable to the low, medium, and high of the others, if the saturation deficit is used as a measure of the degree of humidity.

The temperature in the rooms maintained at 13° C. was hand-controlled by means of brine coils and electric heaters. Water and calcium chloride in evaporating pans were used to maintain the difference in humidity. Both of these humidities when measured by the saturation deficit were lower than any of the humidities employed at the other three temperatures.

In one experiment 10 full-sized green fruits were stored under each of the nine combinations of temperature and humidity at the temperatures 0°, 4.5°, and 10° C. These fruits were examined at frequent intervals for infection with Botrytis rot and anthracnose and for shriveling and ripening of the fruits.

In the second experiment fruits of four types as to size and ripeness were selected, namely, small green (half size), large green (mature green), semiripe, and ripe. Fourteen small green, ten large green, nine semiripe, and ten ripe fruits were stored in 4-quart till baskets at each of the 11 combinations of temperature and humidity given in Table 3. Frequent inspections were made for Botrytis infection, anthracnose infection, shriveling, and the degree of ripening.

TABLE 3.—*Relation of temperature, humidity, and time to infection of pepper fruits by Botrytis*

Temperature	Relative humidity	Fruits used	Number of fruits infected by Botrytis after—						
			11 days	18 days	25 days	32 days	39 days	46 days	53 days
° *C.*	*Per cent*	*Number*							
13	76	43	0	0	0	0	0	------	------
13	71	43	0	0	0	0	0	------	------
10	98	43	0	0	2	3	6	7	------
10	89	43	0	1	1	1	3	4	------
10	84	43	0	0	1	1	1	------	------
4.5	95	43	0	2	3	6	17	20	------
4.5	87	43	0	2	3	3	8	16	------
4.5	74	43	0	0	1	3	5	------	------
0	90	42	0	0	0	2	3	4	19
0	80	43	0	0	0	1	2	4	13
0	69	43	0	0	0	0	1	------	------

The data in the two experiments, with respect to the conditions under which they were conducted, were parallel and correspond so closely that only those from the second experiment will be presented.

Although there was some variation in the number of infections by Botrytis occurring under given conditions at a given time in the various lots, it was not large, and the relation of infection to temperature and humidity in the different lots corresponded closely. For example, the percentage of infection under all of the 11 conditions in 39 days was in ripe fruits 8, in small green 9, in large green 12, and in semiripe 10, and the distribution at the various temperatures and humidities was about the same.

The summary of the results obtained from the four lots is recorded in Table 3. There was no infection at 13° C. in 39 days. Humidity in this case was probably a limiting factor, for infection was obtained in another experiment in a lot stored at 13° and a relative humidity of 92 per cent. It is also possible that temperature may have aided in eliminating infection, for it will be noted that the number of infections occurring at all the relative humidities at 4.5° was markedly higher than at 10°, notwithstanding the fact that the relative humidities at the two temperatures were comparable.

A larger number of infections occurred at all the relative humidities at 4.5° than at 0° C., and the initial infections were observed 14 days earlier.

At each of the temperatures 0°, 4.5°, and 10° C. the higher the humidity the larger was the number of infections, except at 0° after 46 days. Although there was a tendency for the time required for infection to occur to lengthen with the lowering of the relative humidity at a particular temperature, there were exceptions, and the difference in time was never marked.

INFLUENCE OF TEMPERATURE, HUMIDITY, AND TIME ON INFECTION OF PEPPERS WITH ANTHRACNOSE

The data on anthracnose (Table 4) were obtained in connection with study of Botrytis rot, but as they bring out facts of importance in regard to the storage of peppers they are included here. It should be said at the outset that very little anthracnose developed in fruits in any of the experiments aside from the last two now under consideration. In these anthracnose was more conspicuous than Botrytis rot, not only as manifested by the number of fruits infected but by the much larger number of lesions. Anthracnose lesions developed at all the temperatures employed, and at all the humidities except 69 per cent at 0° C., although the fruits were free from visible infection of any kind when stored. There was very little difference in the number of fruits infected at temperatures of 4.5°, 10°, and 13° at any time during the experiment. As the temperature fell to 0° there was a considerable decrease in the number of infections. There was a slight tendency for the time required for the occurrence of the initial infection to lengthen as the temperature was lowered from 13° to 4.5°. The time required for infection to occur was considerably lengthened as the temperature was lowered from 4.5 to 0°.

TABLE 4.—*Influence of temperature, humidity, and time on infection of pepper fruits with anthracnose*

Temperature	Relative humidity	Fruits used	Number of fruits infected after—					
			11 days	18 days	25 days	32 days	39 days	46 days
° *C.*	*Per cent*	*Number*						
13	76	43	1	2	5	7	9	
13	71	43	0	1	3	4	8	
10	98	43	1	1	4	7	12	17
10	89	43	0	1	2	2	7	12
10	84	43	0	0	1	3	15	
45	95	43	0	1	3	7	12	21
45	87	43	0	0	1	5	8	20
45	84	43	0	1	2	9	12	
0	90	42	0	0	0	0	0	2
0	80	43	0	0	0	1	4	5
0	69	43	0	0	0	0	0	

The number of infections that occurred at 13° C. may have been limited by the low humidity employed, although there is little evidence in the table as a whole that humidity was a limiting or modifying influence. At 13° the number of infections was less at 71 per cent relative humidity than at 76 per cent. At temperatures of 4.5° and 10° the smallest number of infections occurred at the medium humidities, the greatest number fluctuating between the high and low humidities with extension of time. At 0° the number of infections was greatest at the medium humidity. The distribution of infection in the individual lots, as in the other experiment, showed the same general relation to temperature and humidity as did the four lots jointly.

It should be kept in mind that the results obtained on the distribution of infection at the various temperatures and humidities may have been influenced to some extent by the distribution of the inoculum, which was contingent upon natural inoculation rather than on a controlled distribution. It is believed, however, that considering the abundance of infection, the distribution must have been fairly uniform.

The following facts may be emphasized: (1) Peppers may become infected with anthracnose, and the disease progresses under the storage conditions employed in these experiments; (2) at a temperature of 0° C. infection is delayed sufficiently to make storage of uninfected fruits, although contaminated with anthracnose spores, feasible for a period of 32 to 39 days.

An examination of the data on the various lots of peppers revealed a marked difference in the amount of infection of small green and large green fruits on the one hand and semiripe and ripe fruits on the other. Nine per cent of the large green fruits and 9 per cent of the small green fruits stored at all the conditions of temperature and humidity became infected by the end of 39 days, while 21 per cent of the semiripe and 28 per cent of the ripe fruits became infected. Barring the possibility that this difference in infection might have resulted from a difference in the amount of inoculum due to a difference in the length of time that fruits remained on the plants, it would seem that these results indicate a greater susceptibility to anthracnose in the semiripe and ripe fruits than in the small and large green fruits.

Dastur [5] claims that the ripe fruits are more susceptible to anthracnose than the green ones.

INFLUENCE OF TEMPERATURE, HUMIDITY, AND TIME ON RIPENING OF PEPPERS IN STORAGE

The results on ripening are recorded in Tables 5 and 6. Table 5 deals with the small green and large green fruits, and Table 6 with the semiripe fruits. The results with the small green and the large green fruits were so similar that they were combined.

TABLE 5.—*Influence of temperature, humidity, and time on ripening of green pepper fruits in storage*

Temperature	Relative humidity	Fruits	Number of fruits showing indicated degree of ripening after—														
			11 days			18 days			25 days			32 days			39 days		
			Just turning	Semiripe	Ripe	Just turning	Semiripe	Ripe	Just turning	Semiripe	Ripe	Just turning	Semiripe	Ripe	Just turning	Semiripe	Ripe
° *C.*	*Per cent*	*No.*															
13	76	24	1	5	0	2	2	4	4	2	5	4	6	5	2	8	6
13	71	24	2	3	0	5	2	3	3	7	4	2	9	4	2	10	4
10	98	24	4	0	0	2	6	0	3	5	1	3	7	2	3	7	3
10	89	24	1	2	0	2	6	0	3	7	1	4	7	2	4	9	2
10	84	24	0	8	0	5	5	4	3	7	5	4	6	7	2	9	7
4.5	95	24	0	0	0	0	0	0	1	0	0	2	0	0	3	0	0
4.5	87	24	2	0	0	3	0	0	2	0	0	4	0	0	1	3	0
4.5	74	24	0	0	0	0	0	0	2	0	0	4	0	0	4	0	0
0	90	23	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	80	24	1	0	0	1	0	0	1	0	0	2	0	0	2	0	0
0	69	24	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

TABLE 6.—*Influence of temperature, humidity, and time on ripening of semiripe pepper fruits during storage*

Temperature	Relative humidity	Fruits	Number of fruits becoming ripe after—				
			11 days	18 days	25 days	32 days	39 days
° *C.*	*Per cent*	*Number*					
13	76	9	7	8	9	----------	----------
13	71	9	3	8	8	9	----------
10	98	9	7	8	8	9	----------
10	89	9	6	7	7	7	7
10	84	9	5	8	8	9	----------
4.5	95	9	0	3	3	4	4
4.5	87	9	1	2	3	2	4
4.5	74	9	0	0	0	0	1
0	90	9	0	0	0	0	0
0	80	9	0	1	1	1	1
0	69	9	0	0	0	0	5

The humidities employed in these experiments seem to have had little or no effect on ripening.

The ripening process went on at all four temperatures (0°, 4.5°, 10°, and 13° C.), but very slowly at 0°, and the rate increased rapidly with the rise in temperature. However, ripening was sufficiently slow in green peppers at both 0° and 4.5° to permit storage for three or four weeks.

[5] DASTUR, J. F. GLOMERELLA CINGULATA (STONEMAN) SPAULD. AND V. SCH. AND ITS CONIDIAL FORMS, GLŒOSPORIUM PIPERATUM E. AND E. AND COLLETOTRICHUM NIGRUM E. AND HALS., ON CHILLIES AND CARICA PAPAYA. Ann. Appl. Biol. 6: 245–268, illus. 1920.

INFLUENCE OF TEMPERATURE, HUMIDITY, AND TIME ON SHRIVELING OF PEPPERS IN STORAGE

At the first sign of softening a pepper fruit was considered shriveled. It was believed that this procedure would result in a more accurate measure of shriveling than any attempt to estimate the degree of shriveling. Since the physical condition of the fruit is more important from the market standpoint than the loss in weight, shriveling rather than loss in weight was used as a measure of loss of water.

With one exception the results, whether considered in connection with the separate lots or jointly, clearly show a marked effect of both temperature and humidity on shriveling. The higher the temperature and the lower the relative humidity, the greater was the number of fruits that shriveled. At a temperature of 4.5° C. and a relative humidity of 74 per cent, the number of fruits that shriveled by the end of 18, 25, and 32 days was greater in all four lots of experiment 2 (Table 7) than at a temperature of 10° and a relative humidity of 84 per cent—although the reverse was true after 11 days—as well as in experiment 1 (data not presented). A humidity of 74 per cent at 4.5° had a slightly greater saturation deficit than a humidity of 84 per cent at 10°, but this fact does not eliminate the difficulty, for there would still be a discrepancy because the effect of temperature is more than offset by this small difference in humidity. Of course, there is some room for error in the method employed in measuring shriveling. On the whole, the results show a marked increase in shriveling with the increase in temperature.

TABLE 7.—*Influence of temperature, humidity, and time on shriveling of green pepper fruits in storage*

Temperature	Relative humidity	Number of fruits used	Number of fruits shriveled after—					
			11 days	18 days	25 days	32 days	39 days	46 days
° C.	*Per cent*							
13	76	43	15	28	36	43	----------	----------
13	71	43	31	42	42	43	----------	----------
10	98	43	1	1	2	8	12	15
10	89	43	5	19	25	32	39	41
10	84	43	19	25	30	38	43	----------
4.5	95	43	0	1	1	5	9	10
4.5	87	43	6	8	18	18	35	39
4.5	74	43	15	32	42	43	----------	----------
0	90	42	0	0	0	0	0	5
0	80	43	0	0	6	6	19	30
0	69	43	0	16	27	42	42	----------

The conditions most favorable for the storage of peppers from the standpoint of shriveling are (1) a temperature of 4.5° C. and a relative humidity of 95 per cent and (2) a temperature of 0° and a relative humidity of 90 per cent. The latter condition was somewhat more favorable than the former.

Taking into consideration the effects of temperature and humidity on infection and development of Botrytis rot and anthracnose and on ripening and shriveling, an examination of the results recorded in Tables 3 to 7 shows that the most favorable condition among those employed for the storage of peppers is a temperature of 0° C. and a relative humidity of 90 per cent. A temperature of 0° was the least favorable of those employed for the development of disease and for

shriveling and ripening. Although relative humidities of 69 and 80 per cent at 0° are less favorable for infection by Botrytis than a relative humidity of 90 per cent, those humidities brought about shriveling too rapidly for the prolonged storage of peppers. The limiting factor at a relative humidity of 90 per cent at 0° is infection by Botrytis, but the earliest infection was observed only after 32 days and the percentage of infection was not large until after 46 days.

SUMMARY AND CONCLUSIONS

Freezing injury and Botrytis rot of peppers are described. The lesions on pepper fruits caused by freezing are shown to be distinct from those produced by *B. cinerea*. The following distinguishing features may be emphasized: The lesions due to freezing injury have a water-soaked appearance, are a darker green than the normal fruit, and are indefinite in outline. The Botrytis lesions, on the other hand, are very definite in outline and are a creamy, buffy olive color.

It has been shown not only that *Botrytis cinerea* is able to infect pepper fruits when used in artificial inoculations, but that it does so normally and commonly at temperatures from 0° to 13° C.

Although peppers stored at temperatures fluctuating from 0° to 2° C. for a few weeks commonly become infected with *Botrytis cinerea*, occasionally there are lots that show little or no infection.

The number of pepper fruits infected by *Botrytis cinerea* out of the lots containing 129, 129, and 128 fruits, stored at temperatures of 0°, 4.5°, and 10° C., respectively, for 39 days, was 6 at 0°, 30 at 4.5°, and 10 at 10°. The temperature of 4.5° was more favorable for infection than either 0° or 10°, notwithstanding the optimum temperature for the growth of *B. cinerea* is about 25°. The time required for the initial infection to occur at 0° was about 32 days, as compared with 18 days at 4.5° and 10°.

There was an increase in the number of Botrytis infections with the increase in the relative humidity at each of the temperatures 0°, 4.5°, and 10° C.

Anthracnose developed on pepper fruits contaminated with anthracnose spores at all the temperatures employed (0° to 13° C.). The time required for infection to occur tended to increase as the temperature fell below 13°.

There was very little difference in the number of anthracnose infections that occurred by the end of the various periods of time employed at temperatures of 4.5° and 10° C. The number of infections found at either of these two temperatures was much larger than at 0°. In general, the number of infections that occurred at 13° was larger than at the lower temperatures, notwithstanding that the two humidities employed at 13° were lower, on the basis of the saturation deficit, than at the lower temperatures. No consistent relation between relative humidity and infection with anthracnose was found at the various temperatures.

Some ripening occurred at temperatures of 0°, 4.5°, 10°, and 13° C. In general, the number of fruits ripening decreased and the time required for ripening increased with the fall of the temperature below 13°. Very little ripening occurred in 39 days in fruits stored at 0° and 4.5°. Humidity did not seem to affect ripening in any way.

In general, the time required for shriveling to occur increased with the fall in temperature below 13° C. At all the temperatures employed, the number of fruits that shriveled after any given period of time increased rapidly with the lowering of the relative humidity. The time required for shriveling to occur decreased with the lowering of the humidity.

A temperature of 0° C. and a relative humidity of 90 per cent were more favorable for the storage of peppers than any of the other temperatures and humidities employed. This temperature is sufficiently above the freezing point of peppers (−1.06°) to insure against injury from freezing.

EXPERIMENTAL STUDIES ON THE GROWTH AND DEVELOPMENT OF STRAWBERRY PLANTS[1]

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INTRODUCTION

The work reported in this paper constitutes a field study of the behavior of several varieties of strawberry plants during the greater part of the growing seasons of 1925 and 1926 at Glenn Dale, Md. Its purpose was to determine the environmental conditions most effective in promoting and limiting plant growth and development under field conditions. The general plan of the studies included detailed measurements of the plants growing in the field, especially of their leaf enlargement, in order to obtain numerical indices of their growth activity throughout the season, together with corresponding records of some of the main influential climatic conditions. The plant data were then compared with the climatic data to find out what correlations existed for the periods studied. The strawberry seems to be particularly adapted to studies on plant activity, because it is usually active from the opening of spring until the arrival of freezing weather, it is easily propagated vegetatively, and it is very tolerant of a wide range of climatic and soil conditions, although it responds with characteristic definiteness and promptness to slight climatic fluctuations. The results of the study show what conditions were most influential in the growth of the strawberry and present a general picture of its development throughout the growing season under Maryland conditions.

CLIMATIC RECORDS

The field records were taken at the United States Plant Field Station at Bell (post office Glenn Dale), Md. The work was begun in 1923 and continued through 1924, 1925, and 1926. As the principal records discussed herein are those of 1925 and 1926, climatic data for these years only are presented. Strawberries are commonly planted at the Bell station about April 1, and top growth usually ceases between October 15 and November 1. The growing season for tops therefore covers 6½ to 7 months, or about 210 days.

Air-and-soil thermograph records, records of white and black spherical porous porcelain atmometers, and records of soil-moisture content were taken in the field near the plants. Additional records were obtained from an official observation station of the United States Weather Bureau located but a few hundred feet from the strawberry field, and sunshine records were obtained from the weather station in Washington, D. C., about 15 miles distant.

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[2] Thanks are due Prof. B. E. Livingston, director of the laboratory of plant physiology of the Johns Hopkins University, for assistance in planning and conducting the experiments.

The atmometers were placed in the plant rows with the spheres from 6 to 10 inches above the ground and somewhat above the foliage. Readings were taken daily or at longer intervals at about 7.30 a. m.

Records of the soil-moisture content were obtained from April to July, 1925, for the most part weekly.[3] Cores were taken in duplicate in each of two spots about 100 feet apart in the plant rows. Each core was divided into three parts to represent three different ranges in depth, 1 to 6 inches, 6 to 12 inches, and 12 to 24 inches. The upper 6 inches had the lowest and the second foot the highest average moisture content for the period for which the records were taken. A marked decrease in moisture content occurred early in June at the period of high temperatures, high evaporation, and high transpiration rates. At no other time was the moisture supply very low, however, and it is not probable that the soil moisture supply at the Bell station was often a limiting factor in the early development of these plants. The moisture content for the upper 6 inches was used for these records.

The sunshine records for the two years correspond closely with the radiation indices, but show minor differences.

PLANT RECORDS

In 1925 nine varieties—Howard 17, Klondike, Missionary, Dunlap, Parsons, New York, Aroma, Sample, and Gandy—were used in the experiments. These include the leading commercially grown varieties, which constitute over 80 per cent of the total strawberry acreage in the United States. Two varieties, Howard 17 and Klondike, were more thoroughly studied than the others, being taken as representative of the northern and of the southern sorts, respectively. In 1926 these two varieties were the only ones used in the studies.

In 1925, 100 plants of each variety were selected for uniformity in size and general appearance and according to the dates when they rooted in 1924. On April 1 they were set in adjoining rows, and after becoming established the 10 plants of each variety at one end of each row were chosen for special study as representative of the 100 of each sort. Conditions were very favorable for the growth of these plants in 1925, and all made satisfactory development.

In the spring of 1926, 100-plant rows of Howard 17 and Klondike were set and a group of 12 plants near the north end of each row was selected for study. Growth was satisfactory but not as rapid in the early part of the season as in 1925.

The plan to obtain plant records at intervals of one week throughout each of the two seasons was generally followed, although some of the intervals were modified in both seasons. At each observation each new leaf that had expanded until most of its upper surface was exposed was numbered and tagged. Likewise, each new runner was numbered and tagged, or removed if that was required by the plan. All leaves and runners were then measured and recorded. The plant records, therefore, showed the complete history of each leaf and runner, including the leaves that developed at the tip of each runner. As runner tips became ready to take root they were pegged down in

[3] The soil-moisture determinations were made by J. W. McLane, assistant biophysicist, Biophysical Laboratory, Bureau of Plant Industry.

position for rooting. In both 1925 and 1926 some of the selected plants were used for a study of their behavior after runners were removed. For this purpose, beginning June 17, 18, or 19 for different plants and continuing to the end of the growing season of 1925, all runners were removed from 5 of the 10 plants of each variety. In 1926 the runners were removed from 10 of the 12 plants, only 2 being allowed to form clons.

The records included leaf measurements, runner measurements, and records of the position of runners. The length and breadth of each of the three leaflets of each leaf and the length of each runner were obtained with a flexible centimeter rule. The length and the breadth were multiplied together to give the leaf product for each leaflet, and the products for the three leaflets were summed to give the leaf product for each leaf. The sum of the leaf product of all leaves gave the leaf product in square centimeters for the entire plant, which is used as an index (I) of its leaf area. To obtain the leaf-product increment for the interval the leaf product at the beginning of an interval was subtracted from that obtained at the end. The mean daily increment in leaf product for an interval was obtained by dividing the leaf-product increment for the interval by the number of days in the interval. Finally, the mean daily percentage increment in leaf product for a period was calculated in two ways, as follows: (1) By dividing the mean daily increment for the interval by the leaf product obtained at the beginning of the interval and multiplying by 100 to give index I_1; (2) by dividing the mean daily increment for the interval by the mean leaf product for the interval and multiplying by 100 to give index I_2. Although the plants grew vigorously, as shown by their indices of leaf area (I) their mean daily increment and the indices I_1 and I_2 are relatively small.

In these studies the mean daily percentage increment in leaf product for any interval (I_2) is taken as an index of plant activity for that interval. The value of this index changes throughout the season, and its fluctuations are very nearly the same as the fluctuations in the rate of production of leaf surface and in the capacity of the plant to produce food. Leaf production is one of the main forms of activity in strawberry plants and may be considered as an index of general activity or growth.

Some special tests were made to determine the relation between these leaf-product values and actual leaf areas. Actual leaf area (by planimeter measurements) and leaf products were obtained for the same leaves of the Howard 17 variety. The results showed that actual leaf area was about 75 per cent of the corresponding leaf-product value with very slight variation. The leaf-product value for the entire leaf was much more satisfactory than was that for the terminal or for either of the lateral leaflets, and the leaf-product value for the terminal was more satisfactory than that of either lateral leaflet or that of the two laterals taken together. It was also found that the leaf product used in the tests was very much more satisfactory than any linear dimension, such as length of the leaflets.

The three indices (I, I_1, and I_2) for each variety and interval from April 1 until June 9 are set forth in Table 1.

TABLE 1.—*Leaf-product indices (I, I_1, and I_2) for nine strawberry varieties, 10 plants of each, by intervals from beginning of the growing season until about the beginning of runner formation, 1925*

Variety	Kind of index	Indices for interval No.—							
		0	1	2	3	4	5	6	7, 8
		Apr. 1–14	Apr. 15–21	Apr. 22–28	Apr. 29–May 5	May 6–12	May 13–20	May 21–26	May 27–June 9
Howard 17	I	53	91	134	168	235	397	578	1,091
	I_1		10.2	6.7	4.6	5.7	8.7	7.6	6.3
	I_2		7.5	5.4	4.0	4.7	6.5	6.4	4.4
Missionary	I	21	44	81	105	166	278		[a] 874
	I_1		15.7	12.0	4.2	8.3	9.0		[a] 10.2
	I_2		10.0	8.4	3.7	6.4	6.3		[a] 5.2
Dunlap	I	18	33	54	68	96	[b] 160		501
	I_1		11.7	9.1	3.7	5.9	[b] 9.5		[c] 9.7
	I_2		8.1	6.8	3.3	4.9	[b] 7.1		[c] 4.7
New York	I	27	45	73	97	159	258		[c] 800
	I_1		9.6	8.9	4.7	9.2	8.9		[c] 9.5
	I_2		7.1	6.8	4.0	6.9	7.1		[c] 4.7
Aroma	I	19	34	57	72	115	162		[d] 415
	I_1		11.1	9.7	3.7	8.5	5.8		[d] 6.8
	I_2		8.1	7.1	3.3	6.5	4.9		[d] 3.8
Gandy	I	19	35	59	79	106	187	[e] 446	589
	I_1		12.1	9.7	4.9	4.9	10.9	[e] 9.7	3.6
	I_2		8.5	7.3	4.2	4.2	7.9	[e] 5.8	3.1
Average of 6 varieties	I_1		11.7	9.4					
	I_2		8.2	7.0					
Klondike	I	37	60	107	144	219	342	493	986
	I_1		8.9	11.2	4.9	7.4	7.0	7.4	7.1
	I_2		6.7	8.0	4.2	5.9	5.4	6.0	4.8
Parsons	I	24	34	59	81	136	227		[c] 728
	I_1		5.8	10.6	5.3	9.8	9.6		[c] 10.0
	I_2		4.9	7.6	4.5	7.2	7.1		[c] 4.8
Sample	I	28	50	96	118	172	243	[e] 468	[f] 749
	I_1		11.1	13.2	3.2	6.5	5.9	[e] 6.5	[f] 6.7
	I_2		8.1	9.0	2.9	5.3	4.9	[e] 4.5	[f] 5.1
Average of 3 varieties	I_1		8.6	11.7					
	I_2		6.6	8.2					
Average of 9 varieties	I_1				4.4	7.4	8.4	8.5	8.3
	I_2				3.8	5.8	6.2	5.7	4.9

[a] For interval May 21 to June 9.
[b] For interval May 13 to 19.
[c] For interval May 20 to June 10.
[d] For interval May 20 to June 11.
[e] For interval May 20 to June 2.
[f] For interval June 3 to June 11.

At the end of the last interval shown in Table 1 (ending June 9, 1925), the 10 plants of each variety were divided into two groups of five plants each and all runners were removed from the plants of one group as they were produced. Measurements were discontinued at different times for the different varieties as shown in Table 2.

TABLE 2.—*Leaf-product indices (I, I_1, and I_2) for nine strawberry varieties, five plants of each with runners left on and five plants of each with runners removed, by intervals from about the beginning of runner formation until the end of observations, 1925*

Variety and group	Kind of index	Indices for interval No.— 9	10	11	12	13	14	15	16	17	18
		June 10–16	June 17–23	June 24–30	July 1–7	July 8–14	July 15–22	July 23–28	July 29–Aug. 6	Aug. 7–19	Aug. 20–Sept. 16
Howard 17											
With runners	*I*	[a]1,346	1,772	2,323	2,885	3,541	4,035	4,781	7,043	10,669	[b]16,551
	I_1	[a]3.2	4.8	4.4	3.5	3.2	1.8	3.1	5.3	4.0	[b]2.1
	I_2	[a]2.2	4.1	3.8	3.1	2.9	1.7	2.8	4.3	3.1	[b]1.6
Without runners	*I*	.....	1,388	1,623	1,879	2,138	2,184	2,375	2,704	2,886	[b]4,590
	I_1	.....	1.6	2.6	2.3	2.0	.3	1.4	1.7	0.5	[b]0.7
	I_2	.....	1.5	2.2	2.1	1.8	.3	1.3	1.4	.5	[b].7
Klondike											
With runners	*I*	1,389	1,772	2,409	2,963	3,682	[c]4,566	[d]5,890	.....	[e]16,762	[b]2,427
	I_1	3.5	4.0	5.1	3.3	3.5	[c]2.6	[d]4.8	.....	[e]6.8	[f]2.1
	I_2	3.1	3.5	4.4	2.9	3.1	[c]2.4	[d]4.2	.....	[e]3.6	[f]1.2
Without runners	*I*	959	1,130	1,362	1,606	1,786	[c]2,097	[d]2,244	.....	[e]2,809	[f]1,775
	I_1	1.7	2.5	2.9	2.6	1.6	[c]1.9	[d]1.2	.....	[e]1.0	[e]−0.5
	I_2	1.6	2.3	2.7	2.3	1.5	[c]1.8	[d]1.0	.....	[e].8	[e]−.6
Missionary											
With runners	*I*	1,095	1,434	1,909	[g]3,253	.....	.....	.....	.....	.....	.....
	I_1	4.7	4.4	4.7	[g]4.4	.....	.....	.....	.....	.....	.....
	I_2	4.1	3.8	4.1	[g]3.3	.....	.....	.....	.....	.....	.....
Without runners	*I*	209	966	1,221	[g]1,682	.....	.....	.....	.....	.....	.....
	I_1	2.3	2.8	3.8	[g]2.4	.....	.....	.....	.....	.....	.....
	I_2	2.1	2.5	3.3	[g]2.0	.....	.....	.....	.....	.....	.....
Dunlap											
With runners	*I*	[h]511	[i]777	1,010	[g]1,916	.....	.....	.....	.....	.....	.....
	I_1	[h]3.7	[i]4.5	4.3	[g]5.6	.....	.....	.....	.....	.....	.....
	I_2	[h]3.3	[i]4.0	3.7	[g]3.9	.....	.....	.....	.....	.....	.....
Without runners	*I*	[h]574	[i]670	827	[g]1,194	.....	.....	.....	.....	.....	.....
	I_1	[h]2.9	[i]2.8	3.4	[g]2.8	.....	.....	.....	.....	.....	.....
	I_2	[h]2.7	[i]2.6	4.0	[g]2.5	.....	.....	.....	.....	.....	.....
Parsons											
With runners	*I*	[h]842	[i]1,087	1,528	.....	.....	.....	.....	.....	.....	.....
	I_1	[h]5.2	[i]4.8	5.8	.....	.....	.....	.....	.....	.....	.....
	I_2	[h]4.4	[i]4.2	4.8	.....	.....	.....	.....	.....	.....	.....
Without runners	*I*	[h]853	[i]1,068	[g]1,194	.....	.....	.....	.....	.....	.....	.....
	I_1	[h]1.7	[i]4.2	3.2	.....	.....	.....	.....	.....	.....	.....
	I_2	[h]1.6	[i]3.7	2.9	.....	.....	.....	.....	.....	.....	.....
New York											
With runners	*I*	[j]917	[k]1,107	[l]1,438	.....	[m]2,567	.....	.....	.....	.....	.....
	I_1	[j]4.2	[k]4.1	[l]3.7	.....	[m]3.2	.....	.....	.....	.....	.....
	I_2	[j]3.6	[k]3.7	[l]3.3	.....	[m]2.4	.....	.....	.....	.....	.....
Without runners	*I*	[j]1,075	[k]1,293	[l]1,452	.....	[m]2,823	.....	.....	.....	.....	.....
	I_1	[j]2.7	[k]4.1	[l]1.5	.....	[m]2.0	.....	.....	.....	.....	.....
	I_2	[j]2.4	[k]3.7	[l]1.4	.....	[m]1.2	.....	.....	.....	.....	.....
Aroma											
With runners	*I*	[n]518	.....	.....	.....	.....	.....	.....	.....	.....	.....
	I_1	[n]4.1	.....	.....	.....	.....	.....	.....	.....	.....	.....
	I_2	[n]3.6	.....	.....	.....	.....	.....	.....	.....	.....	.....
Without runners	*I*	[n]441	.....	.....	.....	.....	.....	.....	.....	.....	.....
	I_1	[n]4.4	.....	.....	.....	.....	.....	.....	.....	.....	.....
	I_2	[n]3.8	.....	.....	.....	.....	.....	.....	.....	.....	.....

[a] Indices for all 10 plants.
[b] Average of 2 plants instead of 5 plants.
[c] For interval July 15 to 23.
[d] For interval July 24 to 30.
[e] For interval July 31 to Aug. 26.
[f] For interval Aug. 27 to Sept. 18 and average of 2 plants instead of 5 plants.
[g] For interval July 1 to 16.
[h] For interval June 11 to 17.
[i] For interval June 18 to 23.
[j] For interval June 11 to 18.
[k] For interval June 19 to 23.
[l] For interval June 24 to July 1.
[m] For interval July 2 to 23.
[n] For interval June 12 to 18.

TABLE 2.—*Leaf-product indices (I, I_1, and I_2) for nine strawberry varieties, five plants of each with runners left on and five plants of each with runners removed, by intervals from about the beginning of runner formation until the end of observations, 1925*—Continued

Variety and group	Kind of index	Indices for interval No.— 9 June 10–16	10 June 17–23	11 June 24–30	12 July 1–7	13 July 8–14	14 July 15–22	15 July 23–28	16 July 29–Aug. 6	17 Aug. 7–19	18 Aug. 20–Sept. 16
Sample											
With runners	*I*	[a] 982									
	I_1	[a] 4.6									
	I_2	[a] 4.0									
Without runners	*I*	[a] 762									
	I_1	[a] 2.1									
	I_2	[a] 1.9									
Gandy											
With runners	*I*	[a] 741									
	I_1	[a] 3.3									
	I_2	[a] 2.9									
Without runners	*I*	[a] 787									
	I_1	[a] 3.8									
	I_2	[a] 3.4									
Average of all varieties											
With runners	I_1	4.4	4.4	4.7	3.4	3.4	2.2	4.0	5.4	5.3	2.5
	I_2	3.7	3.6	4.0	3.0	3.0	2.1	3.5	4.7	3.3	1.6
Without runners	I_1	3.1	2.9	2.9	2.5	1.8	1.2	1.3	1.4	.8	.2
	I_2	2.6	2.7	2.6	2.0	1.6	1.1	1.2	1.2	.7	.1

[a] For interval June 12 to 18.

In 1926 the period of observations extended from June 26 to October 9. The index values obtained are shown in Table 3. Two series of values are shown for each variety, one for the 2 plants with runners (these becoming clons) and the other for the 10 plants without runners.

TABLE 3.—*Leaf-product indices (I, I_1, and I_2) for two strawberry varieties, 2 plants of each with runners left and 10 of each with runners removed, by intervals from about the beginning of the season of runner formation until about the end of the growing season, 1926*

Variety and group	Kind of index	Indices for interval No.— 1 June 26–July 6	2 July 7–12	3 July 13–19	4 July 20–26	5 July 27–Aug. 2	6 Aug. 3–9	7 Aug. 10–16	8 Aug. 17–23	9 Aug. 24–30	10 Aug. 31–Sept. 7	11 Sept. 8–21	12 Sept. 22 to Oct. 7–9
Howard 17													
With runners	*I*	871	1,269	1,526	1,905	2,610	2,899	2,618	3,419	3,934	5,099	8,433	12,954
	I_1	4.7	6.5	2.9	3.5	5.3	1.6	−0.6	3.2	2.2	5.3	4.0	3.3
	I_2	3.7	5.3	2.6	3.2	4.5	1.5	−.6	2.7	2.0	4.6	3.1	2.6
Without runners	*I*	738	1,014	1,108	1,307	1,560	1,673	1,348	1,581	1,586	1,897	2,148	2,655
	I_1	4.3	8.9	1.3	2.4	2.7	1.0	−2.8	2.5	0	2.3	0.9	1.5
	I_2	3.5	7.8	1.3	2.2	2.5	1.0	−3.1	2.3	0	2.1	.9	1.3
Klondike													
With runners	*I*	812	849	1,168	1,287	1,664	1,604	.....	[a] 2,124	2,518	3,308	4,452	5,614
	I_1	3.9	0.7	5.4	1.5	4.2	−0.5	.....	[a] 2.3	2.6	3.9	2.5	1.4
	I_2	3.2	.6	4.2	1.4	3.7	−.5	.....	[a] 2.0	2.4	3.4	2.1	1.2
Without runners	I_1	410	456	635	758	859	845	.....	[a] 788	565	864	940	1,022
	I_1	5.3	1.9	5.6	2.8	1.9	−0.2	.....	[a] −0.5	−4.0	6.6	0.6	0.5
	I_2	4.1	1.8	4.7	2.5	1.8	−.2	.....	[a] −0.5	−4.7	5.2	.6	.5
Average													
With runners	I^1	4.3	3.6	4.2	2.5	4.8	.6	.....	2.8	2.4	4.6	3.3	2.4
	I_2	3.5	3.0	3.4	2.3	4.1	.5	.....	2.4	2.2	4.0	2.6	1.9
Without runners	I_1	4.8	5.4	3.5	2.6	2.3	.4	.....	.7	−2.0	4.5	.8	1.0
	I_2	3.8	4.8	3.0	2.4	2.2	.4	.....	.6	−2.4	3.7	.8	.9

[a] For interval Aug. 10 to 23.

When the intervals in Tables 1, 2, and 3 were the same for all varieties considered, the average index values were derived in the ordinary manner; when the intervals differed so that the averages could not be obtained in the ordinary way, those for the actual intervals used were first plotted as ordinates on a graph with these intervals as abscissas, and the average values were read from the graph for the dates required.

Because the varieties Klondike, Parsons, and Sample behaved differently from the remaining six for the first two intervals of 1925 (Table 1), the averages for these intervals were determined for these three varieties and for the remaining six separately. The two groups behaved similarly throughout the summer after the first two intervals, and for later intervals their index values were combined in single averages and taken to represent both groups together.

The data for climatic values and for leaf-product indices are presented in Figures 1 and 2. Figure 3 shows the appearance of a part of the experimental rows in 1926 with leaves tagged and runner plants pegged.

DISCUSSION

COMPARISON OF LEAF-PRODUCT AND CLIMATIC GRAPHS

A comparison of the plant graphs in the lower parts of Figures 1 and 2 with the climatic graphs in the upper parts of the same figures brings out the fact that the two sets of graphs are not generally in agreement for either season. The seasonal march of the leaf-product indices does not follow the march of any one of the climatic indices here used, but shows the influence of different climatic factors. The first two intervals of 1925 may be disregarded as being especially influenced by genetic differences. The second index (I_2) appears more useful than the first (I_1) and is used in the discussion.

The first elevation of the leaf-product graph for 1925 (intervals 4 to 7) corresponds to temperature, evaporation, radiation, and sunshine-duration values from low to medium, and to high soil-moisture values. The second elevation (interval 16) for the clons, corresponds to a definite depression in the temperature graph and to low values of evaporation, radiation, sunshine duration, and high soil-moisture content. The first elevation of the leaf-product graph for 1926 (interval 5) corresponds to a depression in the temperature-maximum graph, to an elevation in the temperature-minimum graph, and to relative depressions in the graphs for evaporation, radiation, and sunshine duration. The second elevation (interval 10) corresponds to medium values for temperature, evaporation, and radiation, and to a low value for sunshine duration.

The first depression of the graph for 1925 (interval 3) corresponds in time to exceptionally low values for all four temperature indices and to relatively low values for evaporation, radiation, and sunshine duration, but the soil-moisture content for this interval is high. The broad second depression for 1925 (intervals 9 to 15) corresponds to high temperature values throughout, to low or medium soil-moisture values, to high evaporation and radiation values, and to medium sunshine-duration values.

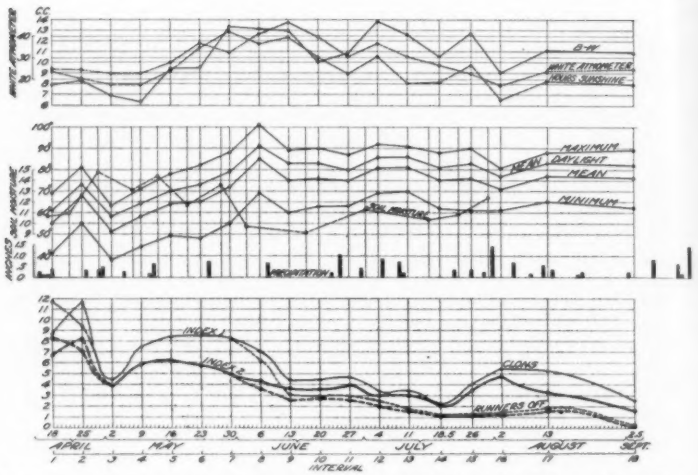

FIGURE 1.—Plant activity shown in terms of leaf-product indices (I_1 and I_2) of the strawberry for 1925 at the United States Plant Field Station, Bell, Md., together with temperature, rainfall, soil-moisture, evaporation, and sunshine graphs for the same period

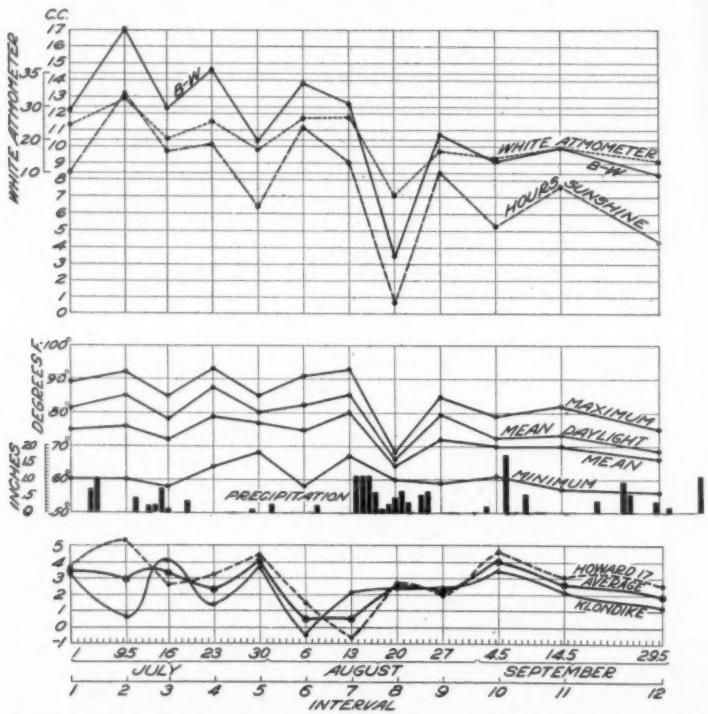

FIGURE 2.—Plant activity shown in terms of leaf-product indices (I_2) in Klondike and Howard 17 strawberry plants and of their average for 1926 with corresponding climatic data

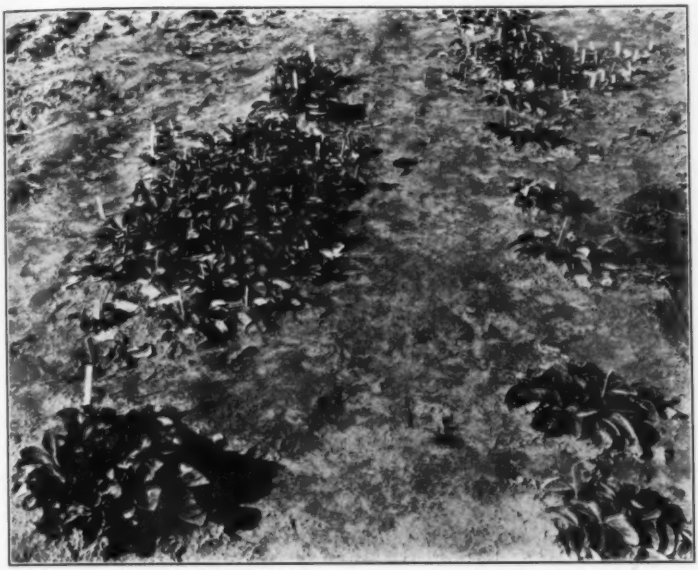

FIGURE 3.—Strawberry plants, Howard 17 (at left) and Klondike (at right), on which growth studies were made in 1926. The plant of Howard 17 in the immediate foreground had all runners removed as fast as formed, while the next plant had its runners pegged to the soil in order to induce them to root promptly

The pronounced depression of the leaf-product graphs for 1926 does not correspond to any of the climatic graphs thus far considered, but it is to be remembered that there is no soil-moisture graph for this season. However, an examination of the precipitation graph shows that this depression of the leaf-product graph represents, in time, the end of a long drought period, and it is safe to suppose that the soil-moisture values at that time were very low.

Considering now only the plant graphs and those for average mean daylight temperature, some indications as to the air-temperature conditions that apparently have been especially favorable or unfavorable for rapid leaf production may be reached. In both seasons high leaf-product values occur for intervals with daylight-temperature values approximately between 64° and 80° F., and low leaf-product values occur for intervals with daylight-temperature values of about 79° or above, and also of 68° or below. These observations, together with many details shown on the graphs, lead to the conclusion that temperature was generally the limiting factor in the production of leaves on the plants used in these studies. In these two seasons the moisture conditions were probably generally adequate, with the exception noted for intervals 6 and 7 of 1926, and no specific influence can be postulated for the conditions of evaporation, radiation, and sunshine duration except in those cases where temperature influence was also indicated. It may therefore be supposed that there was a range of daylight-temperature values from about 64° to 80° within which leaf production went on at high rates, whereas daylight-

temperature values higher or lower than this range gave characteristically low rates of leaf production. It is to be noted that these limits are not exactly the same when derived from the occurrence of high leaf-product values as when derived from the occurrence of low leaf-product values. From these results it might be concluded that the optimum value for the daylight mean is not far from 73° (perhaps between 70° and 76°). This same optimum has been derived by studying these graphs in several other ways, and it may be taken tentatively as approximately correct for the data at hand.

Though plant activity as measured by the leaf-product method indicates low rates below 64° and above 80° F., it should be emphasized that this applies to the plants under the conditions of this study. The responses might be different with the long days in Alaska and the varieties grown there or under irrigation at high elevations in Western States. However, because the varieties included the leading sorts grown in the United States and because of the variety of climatic conditions encountered, it is probable that the activity of tops during most of the growing period responds in a similar manner to these temperatures in the humid regions of the United States. Strawberries are raised successfully in the South, where high temperatures are often encountered during the summer, because there some growth can take place from time to time throughout the long growing season and because of the occurrence of periods with temperatures near the optimum in fall and spring. In the North the strawberry succeeds because the temperatures are near the optimum during much of the summer, even though they are below the optimum during most of the spring and fall.

SEASONAL MARCH OF MATURE-LEAF SIZE AS INDICATED BY MEAN LEAF PRODUCT

The measurement at regular intervals of each leaf on all plants in these studies made it possible to obtain data showing how the leaf size of mature leaves was related to the time of year when they unfolded from the bud, and to their position, whether on parent or runner plants; also, how the production of runner plants influenced mature-leaf size in the mother plants. Four plants of the Howard 17 variety were used for this study. Two were of the group allowed to produce runners and to form clons, and the other two were of the group from which all runners were removed. The leaves were classed according to the dates on which they were first observed unfolding from the bud. After the beginning of runner-plant formation the data for the two plants with runners removed were separated from those for the two producing runner plants. In addition, the runner plants themselves were classed according to the dates on which they were first observed as plants. The data obtained from these leaf measurements were plotted to form the graphs shown in Figure 4.

It will be noted that the graph for mother plants rises at first slowly then rapidly until June 10, when runner plants began to be formed. (Fig. 4.) After June 24, the graph for mother plants with runners (producing runner plants) shows a decided drop, which continues, except for a slight rise July 8, until the end of the period. The graph for mother plants with runners removed maintains its high level, rising from July 1 to July 23. For the period from the

beginning of observation to the beginning of runner-plant formation the leaves of successive leaf classes were progressively larger when enlargement had ceased, that is, the later they unfolded from the bud the larger they became. When runners were removed as they formed, thus preventing the formation of runner plants and the presumable corresponding drain on the mother plant, the leaves of later leaf classes became even larger. But when runner plants were allowed to form, the period of runner-plant formation was characterized by smaller mature leaves on the mother plant. The greatest mean leaf product is for the leaf class of August 7, and its value is 160, which is more than four times as great as the corresponding mean for leaves of the first leaf class (April 15) which had passed the winter in the buds. For the mother plants that were

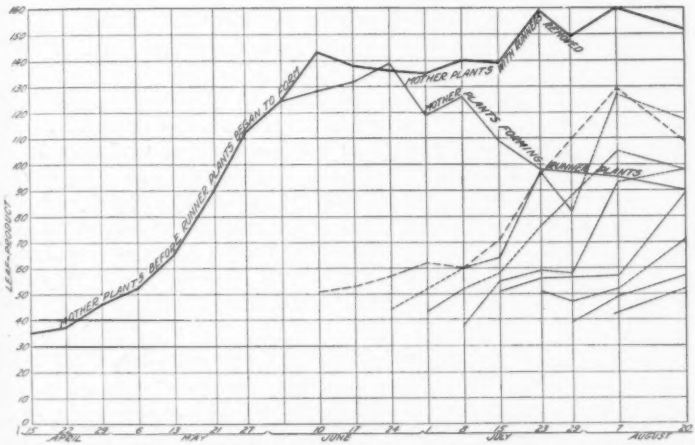

FIGURE 4.—Graph showing (for plants of Howard 17) average size of mature leaves (leaf product) for each leaf class: For mother plants before runner plants began to be formed (broad line before June 10); for mother plants with runners removed (broad line after June 10); and for mother plants forming runner plants (narrow line), as well as for runner plants of each runner-plant class (broken lines). Abscissas represent leaf classes (designated by date on which leaves unfolded from bud) and runner-plant classes (designated by date on which runner plants were formed); ordinates are the average leaf products

allowed to produce runner plants the leaves of the leaf class of August 7 gave a mean leaf product of only 95 when enlargement ceased. Thus, the removal of runners had increased the mean leaf product for this leaf class by more than 50 per cent.

Turning to the graphs (fig. 4) for mean leaf products of leaves on runner plants, it may be noted that the first leaf class in each runner-plant class gave a mean leaf product of the same general order of magnitude as that given by leaves of the first three or four leaf classes of the mother plants, and this seems to be true without reference to the runner-plant class. That is, the first leaves produced by a runner plant apparently attained about the same size when mature, whether the plant took root June 10 or August 7 or at any intervening time, and the size attained was practically the same as that reached by mother-plant leaves that unfolded at the beginning of the season.

This relation is especially striking when it is recalled that the environmental conditions of early spring differed greatly from those of midsummer.

Furthermore, the graphs for successive runner-plant classes closely resemble in shape the graph for the parent plants before runner plants began to form. For any runner-plant class the graph slopes upward, at first slowly and then more rapidly. In the first three runner-plant classes (June 10 and 24 and July 1) a maximum in mature-leaf size is shown for the leaves of the leaf class of August 7, just as in the case of the leaves of the mother plants from which runners were removed. The actual maximum leaf products for the first two runner-plant classes (June 10 and 24) are much smaller (129 and 127) than the corresponding value for the plants with runners removed (160), but much greater than that for the mother plants producing runner plants (95). The maximum for the third runner-plant class (July 1) is smaller, but still greater than the mother-plant value just mentioned. The succeeding five runner-plant classes each gave progressively larger mature leaves with successive leaf classes, but apparently the growing season was not long enough to allow the graphs of these runner-plant classes to show maxima. At any rate, for these last five runner-plant classes, leaves of the leaf class of August 20 gave higher mean leaf products at maturity than did those of the leaf class of August 7.

It seems to be indicated that under the conditions of this test, with apparently ample moisture, the size of a runner-plant leaf at maturity was determined largely by the age of the runner plant at the time the leaf unfolded, rather than by the special environmental conditions prevailing either at the time the runner plant rooted, at the time the leaf unfolded, or during the period of enlargement of the leaf.

From the results of this study of mature-leaf size the following picture of some aspects of the development of the strawberry plants dealt with may be tentatively presented. The early developmental stages of the spring-set plant consist in a rapid enlargement of the leaf and root systems by which the rate of net income in all necessary materials is rapidly increased; enlargement of leaf and root systems and the general maintenance of health cost the plant a considerable quantity of essential materials, but the increased income more than offsets these costs from a very early period of development. A larger root system results in a more rapid supply of water and salts from the soil, and a larger leaf system results in a more rapid production of the products of photosynthesis. Leaves that unfold later are therefore better supplied with needed materials than are those that unfold earlier. The later ones should therefore approach the genetic maximum leaf size for the variety considered. But the production of runners and runner plants soon begins, and these are more active in growth than the crown of the parent plant, apparently actually decreasing its supply of necessary materials and limiting the enlargement of leaves that unfold from the crown after runner formation has set in. For a time the runner plant seems to hold the advantage, and its leaves become larger than those contemporaneously developed from the crown, thus approaching the maximum leaf size for the given variety. In its early stages of development the runner plant receives from the parent crown relatively large quantities of water, salts, and the products of photosynthesis. It soon forms, in addition, large

quantities of the products in its own rapidly enlarging leaf system, and derives additional water and salts through its own root system, especially since its roots enter regions of soil not previously explored by the roots of the parent plant.

In turn, the runners and runner plants produced by the first runner plants to be formed from a mother plant may indirectly affect the mother plant itself to some extent, and their relations to the first runner plants are in general the same as those that obtain between the original crown and the first runner plants.

LONGEVITY OF LEAVES AS RELATED TO TIME OF UNFOLDING

Although the strawberry is an evergreen plant, at the Bell station its individual leaves do not live even throughout the growing season. The first leaves to die are those that passed the winter in the expanded mature condition and the next are those that were partially developed in the preceding fall and passed the winter in the bud. Leaves produced entirely in the spring live longer than either overwintering kind.

To study the relative length of life of leaves that unfold at different times in the growing season, data derived from the five plants of the Howard 17 variety with runners removed were employed. The leaves were serially numbered, the length of life was determined for each leaf, and the resulting values were grouped by leaf classes and by length of life in each class. These data include 12 leaf classes from April 15 to July 8, 1925, for all five plants grouped together. Each class includes all leaves first observed as unfolded on the date representing their class. In any leaf class there was considerable variability in leaf longevity, but all leaves of each of the first seven classes (April 15 to May 27) were observed to have died by August 6. Some leaves in each of the later classes were still alive on August 10 when observations on time of death were discontinued.

The results obtained are presented in Figure 5. Approximate dates of unfolding and of death are shown as abscissas, and the serial numbers of the 118 leaves that were studied are shown as ordinates. The height of the shaded blocks at the left-hand end indicates the serial numbers of the leaves and consequently the total number in each leaf class. Each block is bounded at the right by a stepped line the vertical portions of which show the approximate dates of death of the corresponding leaves. The lowest serial leaf numbers in each class refer to the shortest lived leaves of the class. For example, 10 leaves were observed as unfolded on April 15, and these are serially numbered from 1 to 10; therefore the leaf class of April 15 included leaves Nos. 1 to 10. Eight of these (Nos. 1 to 8) were recorded as dead on June 10 and the remaining two (Nos. 9 and 10) on June 17. The second leaf class included six leaves (Nos. 11 to 16). Of these, one died June 10, three June 17, one June 24, and the remaining one July 1. The blocks for later leaf classes may be interpreted in a similar manner by reference to the marginal scales. Of the 118 leaves studied, 87 had died by August 19. Their length of life ranged from 21 to 77 days and averaged 56 days. It may therefore be said that the mean or normal longevity of these summer leaves was nearly two months.

In a general way the leaves died in sequence, those of the earlier classes dying first; but exceptions will be noted, doubtless owing to peculiar conditions operating in these cases. It is not possible to state

just why some leaves of a given class died before others of the same class. Leaf-spot diseases (caused by infection by Macrospirella or Dendrophoma) may have been a contributing factor, but general observation led to the conclusion that infection was not generally important in determining the dates of death of an infected leaf. Infection was not usually observed until the leaves were mature or past maturity. Old leaves may have offered exceptionally favorable conditions for infection. The appearance of these leaf spots almost exclusively on older leaves can hardly be related to a more or less fixed period necessary for the preliminary development of the fungus within the tissues, for other varieties susceptible to these diseases showed severe injury within a few days after the leaves unfolded.

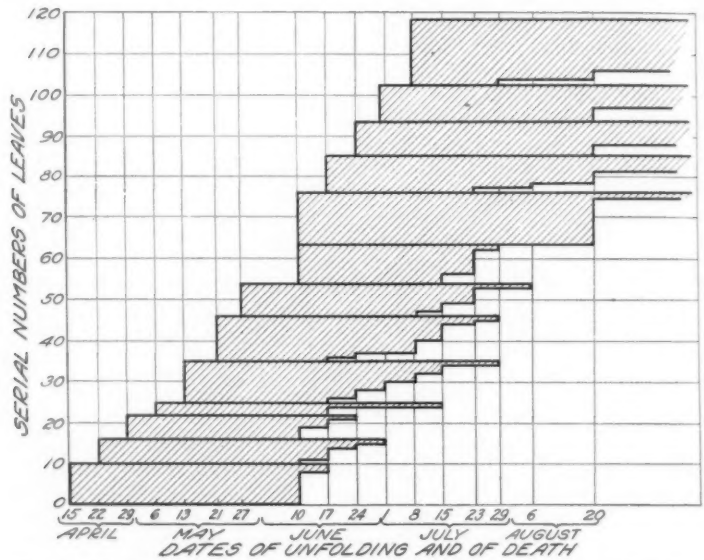

FIGURE 5.—Length of life of all leaves produced by five plants of the Howard 17 strawberry with runners removed, from April 1 to July 8, 1925

COMPARISON OF VARIETIES WITH RESPECT TO NET LEAF PRODUCTION

Seven different varieties were compared with respect to the seasonal march of the value of the average total leaf product per plant, and the results are shown graphically in Figure 6, the data for which are derived from Tables 1 and 2. Ten plants of each variety, together with their runner plants, were used in obtaining the average values. The scale of values of the average total leaf product per plant is shown at the left, and the progress of the season (from April 15 to July 1, 1925) is shown by points and dates along the base line.

For all varieties development is seen to have been slow until about May 13, after which net leaf production was more rapid. In general the relative position of the several lines remains the same throughout their course; varieties with larger values at the beginning of this period had larger values throughout, for there is little crossing of the

lines of the figures. The most active variety was Howard 17 and the least active was Aroma. The latter generally makes but poor growth on the sandy soil of Bell station.

COMPARISON OF NET LEAF PRODUCTION IN PLANTS WITH RUNNERS REMOVED AND WITH RUNNERS LEFT ON AND ALLOWED TO ROOT

The seasonal march of the average value of the total leaf product per plant was studied for the period from April 15 to August 20, 1925,

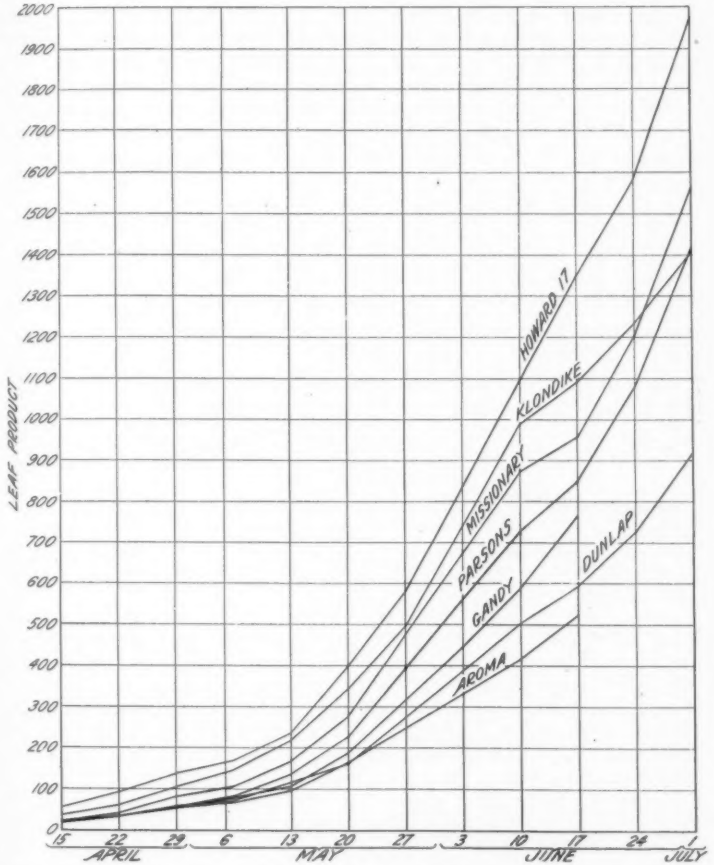

FIGURE 6.—Seasonal marches of total leaf area per plant (as indicated by average values of total leaf product per plant) for each of seven different varieties of strawberry. Ten plants with their runner plants were used for each variety, and all were set out April 1, 1925

for plants with runners removed and with runners left and allowed to produce runner plants. In the latter case the runner-plant leaves were not included. Five plants were included in each group. The average values (Tables 1 and 2) are shown graphically in Figure 7 by the continuous line marked "Runners off average," representing

those from which runners were removed as formed. In this figure are shown also narrow-line graphs for the minimum value of the leaf product and for the maximum value corresponding to the two main graphs. These minimum and maximum values are not shown in Tables 1 and 2, but have been calculated from the original observational data.

It is seen that the average rate was slow till about May 13, after which it was more rapid but nearly uniform to the end of the period in the case of the runnerless plants, but only until about July 1 for the plants that were allowed to produce runner plants. After about July 15 the average graph for the latter group of plants descends slightly and then remains nearly horizontal to August 20, when these observations were discontinued. It is thus again indicated that the vigor of the parent plant was greatly increased by the removal of the runners as they appeared. In other words, the production of runners and runner plants limits the growth vigor of the parent plant through-

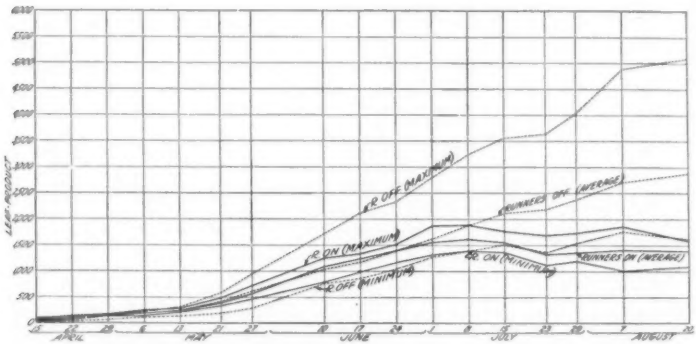

FIGURE 7.—Seasonal marches of total leaf area per plant (as indicated by values of total leaf product per plant) for plants of the Howard 17 variety of strawberry, with runners removed and with runners left and allowed to root. Five plants were included in each group; all were set out April 1, 1925

out the latter part of the season. On August 20 the average value of the total leaf product per plant of the plants without runners was nearly twice as great as that of the plants with runners.

It is to be noted, however, that the maximum deviation from the average is greater for the plants with runners removed than for the others, and that this difference becomes greater with the advance of the season.

COMPARISON OF NET MEAN DAILY INCREMENTS OF MEAN LEAF-PRODUCT VALUES FOR PLANTS WITH RUNNERS REMOVED AND WITH RUNNERS RETAINED

The seasonal fluctuations in the value of the mean daily increment of leaf production were determined in terms of the corresponding leaf-product increments for the period from April 1 to August 20, 1925. The data for these daily increments were derived from those given in Tables 1 and 2 for the plants of Howard 17. Ten plants were used before June 17, after which five plants with runners were used and five with runners removed. The results are presented in Figure 8.

For the first four intervals (April 1 to May 6) the value of the daily increment was about five, or nearly constant. For the next four intervals (May 6 to June 10) it increased until a maximum of 34 was reached for the last of these four intervals. From the interval beginning June 17 to that beginning July 15 the plants with runners removed showed steadily decreasing increment values until these values became negative (indicating net losses of leaf area) for the intervals beginning July 8 and July 15. These plants showed small positive values for the next two intervals and a small negative value for the interval beginning August 7.

The plants with runners gave positive increment values for all intervals, with much fluctuation. The values are exceptionally low for the intervals beginning July 15 and August 7.

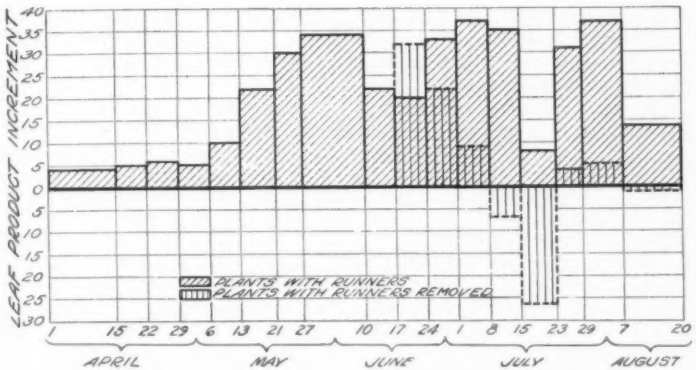

FIGURE 8.—Mean daily increments of the average value of the total leaf product per plant for each observational interval from April 1 to August 20, 1925. Before June 17, 10 plants were employed; for the rest of the period 5 plants without runners and 5 with runners were used, the two sets being treated separately

COMPARISON OF AVERAGE TOTAL LEAF-PRODUCT VALUES FOR CLONS WITH CORRESPONDING VALUES FOR THEIR MOTHER PLANTS

For the five clons of the Howard 17 variety in 1925 the seasonal march of the total leaf surface per entire clon was compared with the corresponding march of the total leaf surface per mother plant in the clon, leaf-product values being used as indicators of leaf surface. The results are shown in Figure 9. The deviation from the average is seen to be small throughout the period, becoming larger for the later dates. The graph of the averages for mother plants alone is not far from the horizontal line for 1,500, showing again that the production of runner plants soon put a stop to the regular increase in net leaf surface of mother plants that was manifest for the first three intervals considered, beginning May 13, 21, and 27. The lined area between the two average graphs represents for each interval the portion of the total leaf product per clon that was due to leaves borne by the runner plants of the clon.

By July 8 the total leaf area of the runner plants of a clon nearly equaled that of the mother plant, and by August 20 the runner-plant portion of the clonal leaf area was nearly seven times as great as the mother-plant portion.

SUMMARY

This paper reports some of the results of a series of field studies on the relations between the growth of strawberry plants and the climatic conditions throughout the growing season. As an index of growth the leaf product (which is the sum of the products of the length and breadth for all leaflets on the plant) was used, and this was found to be a better growth-index than the leaf product for the terminal or for either of the lateral leaflets or for any linear dimension.

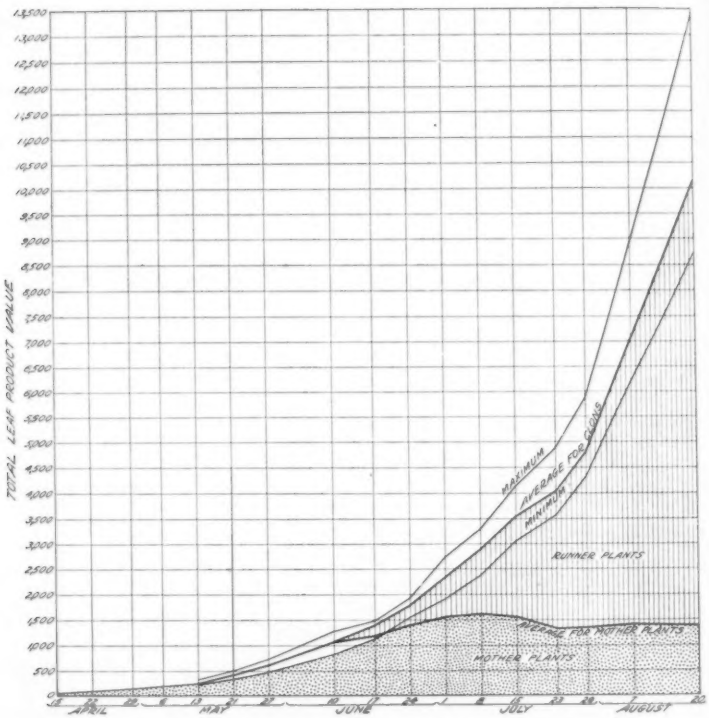

FIGURE 9.—Seasonal march of total leaf area per clon (as indicated by values of the total leaf product per clon) for five clons of Howard 17 variety, season of 1925; also the corresponding graph of the average total leaf product for the mother plant. The shaded area represents the runner-plant portion of the average total leaf product per clon at each time of observation

Measurements were taken at intervals, generally of one week, and seasonal graphs were constructed to represent the march of the leaf-product index throughout the season. Some of the climatic conditions were measured at like intervals, and graphs for these were also constructed.

A comparative study of these graphs with special reference to periods of high and low rates of leaf production leads to the conclusion that for the seasons 1925 and 1926 the limiting factor in the growth of the plants was generally temperature. In one instance, however,

low growth rates appear to have been due to drought. The temperature relations are considered in some detail, and the conclusion is reached that these data indicate high growth rates for average mean daylight air temperatures from about 68° to about 79° F., while daylight-temperature values above or below this range were generally accompanied by much lower rates of leaf production. These considerations lead to the approximation of a daylight-temperature optimum of about 73°.

It is emphasized that the results of these studies are to be regarded as valid only for the particular plants and seasonal and other environmental conditions that were involved in this work, and no generalizations are attempted.

Attention is called to the seasonal march of average mature-leaf size on spring-set plants with a seasonal maximum in June. Following the maximum, plants producing runners showed a decline in the size of their mature leaves for the remainder of the season, whereas plants with runners removed showed no such decline. This seasonal march for runner plants also showed an initial minimum, a later maximum, and a final decline. For the Howard 17 variety the average length of life of plants that unfolded between April 1 and July 8, 1925, was 56 days, with a range from 21 to 77 days. In general, the leaves died in sequence, those unfolding first being the first to die.

Plants allowed to produce runners showed a gradual increase in total leaf product until about July 8, after which this measure of leaf area decreased, but only to a slight extent for the rest of the period. By July 8 the total leaf product of the runner plants produced equaled that of the mother plants, and by August 20 it was about seven times as great.

A comparison of the seasonal march of the value of the average total leaf product per plant for 1925 for seven varieties shows that Howard 17 was the most active and Aroma the least active variety. Net leaf production by plants with runners removed was compared with that by plants with runners left on and allowed to root. The vigor of the parent plant was greatly increased by the removal of runners as they appeared, but the maximum deviation from the average was greater for these plants than for those that produced runner plants. A similar comparison was made between the mean daily increment values of total leaf product for the same plants. For plants with runners removed these values were all positive, although with much fluctuation; whereas for the plants producing runners they were generally much smaller and were actually negative (indicating a net loss) for 3 of the 17 periods.

A STUDY OF SOME UNPRODUCTIVE CHERRY TREES IN CALIFORNIA[1]

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INTRODUCTION

Observations made in California over a period of years have disclosed an interesting problem regarding unproductive cherry trees. An occasional tree, or portion of a tree, has been found that produces practically no fruit, yet branches of the same tree, or other trees of the same variety standing beside it, have been consistently prolific. The behavior of these unproductive trees suggests the presence of a virus disease such as mosaic, but the results of the studies thus far conducted indicate that from a commercial standpoint they may be treated as a result of bud variation. The Black Tartarian is the only variety of cherry widely grown in California in which the occurrence of this unproductive strain is frequent. In orchards of this variety it is common to find 1 tree in 10 made up entirely or largely of the unfruitful type of wood, and much higher percentages have been noted. Occasional trees of the Bing, Lambert, Black Eagle, and other varieties have been found with leaves much like those of the unproductive Black Tartarian. Affected trees of these varieties have been found bearing considerable fruit, but it is misshapen and not marketable.

The conditions under which the productive and unfruitful Black Tartarian trees are growing appear to be identical, and in some instances the two types of trees are so close together that their branches overlap. (Fig. 1.) Trees of the unproductive strain have been found in all the orchards in central California where a search for them has been made, and they have also been found in southern California and northern Oregon. Not all cherry-growing localities in these latter districts have been searched carefully for affected trees.

The presence of 7 trees that were made up entirely or almost entirely of unproductive branches was recorded in one orchard in 1924, 17 in another orchard in 1925, and an additional 94 in a third orchard in 1926. In these orchards there are, respectively, 200, 190, and 980 trees of the Black Tartarian variety. A number of the trees that failed to fruit had been top grafted or removed from the first-mentioned orchard before these records were made. Many trees of the same type have been observed in other orchards. None of the affected trees or branches examined have improved and become productive, although the condition has become more noticeable on some trees with the growth of the affected branches.

[1] Received for publication Apr. 11, 1930; issued September, 1930.

LEAF SYMPTOMS

The shape and color of leaves on the unproductive trees differ from those on productive trees, the blossoms are defective, and many blossom buds fail to open. "Curly leaf trees," "wild trees," and "male trees" are terms frequently used by growers to describe the unfruitful strain. The leaves produced by these trees have large or small areas that are somewhat dwarfed, and this dwarfed condition sometimes causes pockets or drawn places in the leaves which give them a wrinkled appearance. To one familiar with these irregularities, the affected leaves present an unnatural appearance even from a distance of two tree spaces. Affected leaves are distinctive in shape. (Fig. 2.) The margins are very irregular, due to the lack of development of

FIGURE 1.—Productive (right and left) and unproductive (center) Black Tartarian cherry trees. All three of the trees are of the same age and have been given the same treatment

some part of the leaf. This dwarfing may affect the end or part of the end of the leaf, the side or part of the side, or the entire leaf. The dwarfing may vary considerably in severity in different parts of the leaf. The dwarfed places range in size from small spots to areas that include almost the entire leaf. Sometimes they appear as narrow strips instead of spots and extend out from the midrib. Many leaves have more than one, and occasionally there are several dwarfed places in a single leaf. Sometimes only a small segment of the leaf fails to develop normally, causing a notch in the margin. This dwarfed condition is often more pronounced along one side of the leaf than the other, and sometimes one side fails almost entirely to develop while the other side appears to be normal.

In color the leaves of the unproductive trees differ from those of the normal leaf. With the unproductive tree there is a characteristic mottling, as the dwarfed areas are a lighter green than the remainder

of the leaf. The margins of the light-green areas are not always well defined around the entire periphery, and sometimes the spots fuse with one another or gradually disappear into the darker green portion of the leaf. These light-green areas are present when the leaf first unfolds in the spring, and they appear to result from the lack of development of chlorophyll rather than from discoloration. Outside the light-colored area the leaf is often a darker green than the normal Black Tartarian leaf. The two extremes of color and the dwarfing of the light-green areas of the leaf are sufficiently striking to enable an experienced observer to recognize the unproductive tree readily.

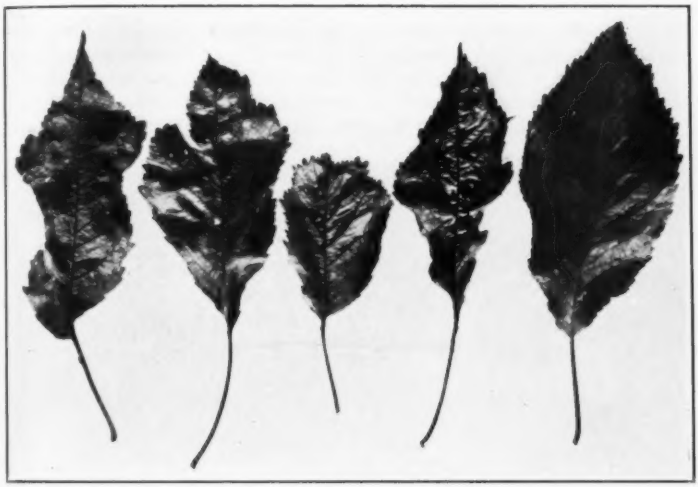

FIGURE 2.—Four malformed leaves (left) from an unproductive Black Tartarian cherry tree and one normal leaf (right) from a thrifty tree in the same orchard

The failure of the leaf, or a part of it, to reach normal size results in a much lower average leaf area on the unfruitful trees than on the normally fruitful ones. Measurements of leaves taken from a 15-year-old tree which has borne almost no fruit and where the malformation is thought to be about average for unproductive trees show that the average leaf area is only three-fourths that of a normal tree standing beside it. On some of the less vigorous of the unproductive trees the leaves appear to roll more during dry weather than those of productive trees, while leaves of the more vigorous ones appear to roll less. The effect of drought on the leaves seems to be determined rather by the vigor of the tree than by the trouble that causes the abnormal development of the leaves.

The extent of mottling and the irregularity in the shape of the leaf of the unproductive strain are sometimes less pronounced on the leaves of shoots that are produced during the summer than on those grown from the buds that open in the spring. Instances have been noted where leaves that grew during the summer were affected but

little if any, whereas all leaves of the same tree that grew early in the spring and in the spring following were decidedly abnormal.

At least an occasional affected leaf was found on the majority of the Black Tartarian trees examined, although on many of these trees no other trace of the malady was apparent. Sometimes the affection was limited to only a few leaves or to the leaves on an occasional spur or branch, and in other cases it was displayed over much of the tree. On the so-called unproductive trees normal leaves and normal fruits could also be found. Some Black Tartarian trees appeared to be entirely free from the off-type leaves.

GROWTH AND BLOSSOMING

In orchards where observations have been made, including those not yet in bearing and others up to more than 20 years of age, there appears to be no difference in the rate of growth between the normal and the unproductive trees. Trees of both types standing side by side have been observed, but no consistent difference in size of trees of

FIGURE 3.—Exposed pistils of blossom clusters of productive (one at right) and unproductive Black Tartarian cherry trees

the same age has been noted. Occasionally a tree of the unproductive strain is larger than its neighbor, and sometimes smaller, but usually it is about the same size. In one orchard, which contains 190 trees of the Black Tartarian variety, the largest tree is made up mostly of affected branches.

The unfruitfulness of the trees under discussion is not due to their failure to blossom, for they bloom profusely and at the same time as the productive trees. Many of the blossoms are defective, however, and smaller than those of normal trees. The peduncles are short, the petals small, and the pistils short and slender with a tendency to discolor early. Many of the pistils fail almost entirely to make any growth. (Fig. 3.) Among the discolored pistils some turn brown before the blossoms open, some soon afterwards, while some retain their normal color for a few days. Now and then a pistil is observed that appears to develop like those on fruitful trees. An occasional fruit is found on unproductive trees, but it is small, rough, and ill shaped. (Fig. 4.)

Although the unfruitful trees blossom profusely, some of the blossom buds that are formed fail to open. The buds at and near the terminals of spurs and shoots produce their blossoms, while those nearest the base of the previous year's growth often fail to open fully. (Fig. 5.) These buds usually swell but stop growth about the time the scales open and the green-bud clusters appear. The swollen buds turn brown and become dry, but often remain in place on the shoot or spur for several weeks.

SOIL AND CULTURAL CONDITIONS

In some orchards where unproductive trees have been under observation there is considerable variation in thrift and production among the trees due to soil or cultural conditions, but in none of the cases observed can the peculiarities of the unproductive trees be attributed to these causes. In some orchards the accumulation of soil moisture during part of the year is responsible for considerable variation in the

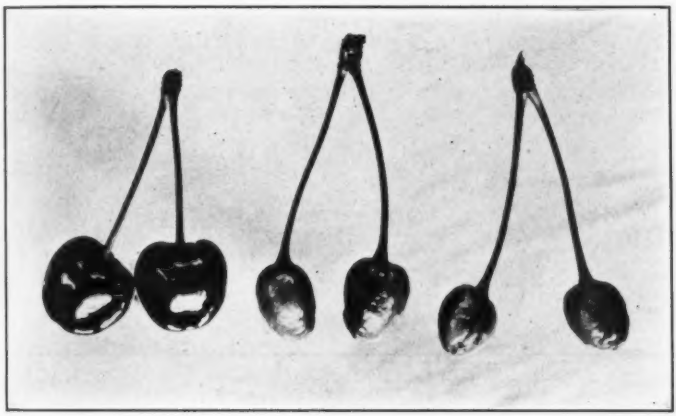

FIGURE 4.—Clusters of cherries from normal (left) and unproductive (two at right) Black Tartarian cherry trees

thrift of the trees. In these orchards a stratum of hardpan underlies the surface soil at a depth of from 2 to several feet, preventing a free filtration of water. This results in an accumulation of soil moisture during the winter months when rainfall is usually plentiful, and occasionally also after summer irrigations. In these places where conditions for growth are unfavorable some trees have died, others have made but little growth, and the yield of fruit and size of leaves have been below normal. The peculiarities of the unproductive trees under discussion are not, however, such as are found in these wet places, nor are they the symptoms commonly found in soils unsuitable to root growth. Trees of the unproductive strain are not more numerous in such places than where satisfactory conditions for growth prevail.

In a study of the distribution of unproductive trees throughout the orchards there was no indication that the local environment was responsible for their behavior. A few of these trees were found more or less grouped in different parts of the orchards, but as frequently

they were scattered singly over large orchards. In some orchards only an occasional unproductive tree was found; in others they were numerous.

In a few of the orchards under observation a number of trees have been injured to such an extent by the application of stable manure that many of their branches have died. The annual growth on these trees has been very short, and the leaves are small. Many of the leaves on the upper and outer branches are light-green or yellowish in color. The unthrifty appearance of these trees differs, however, from that of the unproductive strain, and none of the abnormal characteristics of the unfruitful trees are found on those injured by the manure. Some of the unproductive trees studied are in orchards that have been given manure, and others are in orchards that have received none.

FIGURE 5.—A branch of an unproductive Black Tartarian cherry tree on which the flower buds below the terminals of the shoots and spurs failed to open

EFFECT OF TOP GRAFTING AND BUDDING

A number of trees of the unproductive strain which were recognized by growers have been either removed or top grafted with scions from productive trees, both of the Black Tartarian and of other varieties. This explains why there are not more of them found in some Black Tartarian orchards. In all instances observed the new tree tops which followed grafting have been normally productive and free from any of the malformation of leaves, blossoms, or fruit common to the off-type trees. Where shoots have been allowed to grow from the portion of the branches that remained after the tree was headed back for grafting, the leaves produced by them have manifested the same irregularities in shape and color that were found on the tree before it was headed back, although the growth from the scions has been healthy and productive. Shoots which grow from recently cut-back unproductive trees make a rampant growth, and their leaves develop to a large size, but this has not prevented the development of the characteristics of the unproductive tree in them.

For experimental purposes a few trees of the unproductive strain of the Black Tartarian variety were top grafted in 1928 with scions from both productive and unproductive Black Tartarian trees, both types of scions being inserted into the same tree. Several branch stubs in each of a number of trees were grafted in this way. As the scions grew all those from the productive trees produced normal leaves, and all those from the unproductive trees displayed the same characteristics as the tree from which they were taken. Neither in their first nor second year's growth have the leaves of any of the scions changed in their habit of growth, but they are the same in appearance as those of the parent trees.

In the spring of 1929 the scions from productive trees produced numerous blossoms, all of which appeared to be normal and set fruit of normal shape which grew to maturity. Many of the flower buds of the scions from the unproductive trees failed to open, and many of the blossoms which opened were small, had short unthrifty pistils, and none of them set fruit. A number of branches were allowed to remain on the top-grafted tree when its branches were cut back for grafting; and as the trees were headed back at a height of about 5 feet, numerous small branches and spurs are still growing on the stock portion of the tree. Neither the pruning done in heading the tree back nor the top grafting has affected the habits of these remaining branches. They are unproductive, and the leaves and blossoms are of the same unthrifty type as those produced before the branches were headed.

Attempts were made to transmit the faults of the unproductive strain by budding. Buds from this strain of Black Tartarian were inserted in nursery seedlings in the spring of 1928. The growth from these buds exhibited the same faults as the trees from which the buds were taken, while that on the check trees was normal.

UNSUCCESSFUL ATTEMPTS AT TRANSMISSION BY OTHER MEANS

Efforts to transmit by other means the abnormal development in unfruitful trees have been unsuccessful. Branches of fruitful and unfruitful trees growing side by side have been fastened together in such a way that the leaves would rub one another as the branches were moved by the wind; juice from affected leaves of different ages has been expressed and immediately applied to healthy leaves of different ages, some of which had been pierced and some just slightly scratched to break the epidermis; healthy and affected leaves on adjoining branches have been pinned together and left remaining on these branches; but no indication has been observed that any of the leaf symptoms that accompany unproductiveness have been transmitted.

A number of instances where fruitful and unproductive branches are growing side by side on the same tree have been under observation for three successive years. (Fig. 6.) During this period the performance of none of the branches has changed. The unproductive branches, with their unthrifty leaves, have remained the same, while the productive branches have been healthy and prolific. There is no indication that the position of the branches on the tree influences their behavior.

The occurrence and prevalence of off-type leaves and unfruitful branches in the varieties of cherries studied resembles the condition found in some of the common variegated plants on which occasional normal leaves, twigs, and branches occur.

FIGURE 6.—Unproductive (left) and productive (right) branches growing side by side on the same Black Tartarian cherry tree

PREVENTIVE AND REMEDIAL MEASURES

From the observations thus far made it appears that the unproductive trees are a strain of a variety that has resulted from the propagation of "sporting" branches or "variegations," and if such is the

case the trouble may be evaded by care in the choice of propagating wood. Since the irregularities in the leaves are less noticeable in the fall than earlier in the season, and leaves of shoots that are produced during the summer often display only a trace of the trouble, the location and labeling of propagating wood may be most surely and easily done before the fruit is harvested.

Whatever may be the cause of the trouble that results in the failure of the trees to fruit, the removal, by pruning, of affected branches and by top grafting where the entire tree or a considerable portion of it is affected are effective remedial measures. The selection of propagating wood from productive trees appears to be a satisfactory preventive measure.

THE EFFECT OF HYDROGEN-ION CONCENTRATION ON THE TOXICITY OF NICOTINE, PYRIDINE, AND METHYLPYRROLIDINE TO MOSQUITO LARVAE [1]

By CHARLES H. RICHARDSON,[2] *Entomologist*, and HAROLD H. SHEPARD, *Assistant Entomologist, Division of Deciduous-Fruit Insects, Bureau of Entomology, United States Department of Agriculture*

INTRODUCTION

Previous investigations of the toxicity to insects of nicotine in aqueous solution have involved principally a study of the effects of its application as a spray or dip. The solutions employed have generally contained nicotine sulphate and soap or other alkaline substance, the purpose of the latter being to increase the wetting and spreading properties of the liquid and to convert the nicotine salt into the uncombined, volatile base. After the application, the insect is in contact with continuously varying quantities of nicotine in solution and nicotine gas as evaporation of the alkaloid and water progresses. Under these conditions the toxic effect of nicotine has been explained as the result of its entrance in the gaseous state through the spiracles into the tracheal system of the insect.

In addition to the variable concentration, the results of treating insects by the method just mentioned are complicated by the presence of the alkaloid in three conditions, viz, as gas molecules, as molecules in solution, and as ions. It seemed probable that additional light could be thrown upon the nature of the effect of nicotine upon insects if gaseous nicotine could be eliminated, leaving only the molecules and ions in solution to act upon them. Then, by varying the hydrogen-ion concentration, the insects could be subjected to solutions differing widely in relative content of dissolved molecules and ions.

Accordingly, an aquatic insect, the larva of the house mosquito (*Culex pipiens* L.) was chosen for the purpose and proved to be an excellent test insect.

EXPERIMENTAL PROCEDURE

MATERIALS AND METHODS

The mosquito larvae were reared in a greenhouse in battery jars 15 cm. in diameter. Each of the jars contained approximately 3 liters of tap water in which a quantity of soil and decayed leaves was suspended. Within 24 hours most of the solid matter fell to the bottom of the jars, leaving a solution rich in food for the larvae. Female mosquitoes entered the greenhouse on warm nights and oviposited freely on the surface of the water in the jars. Each morning the egg boats were collected and distributed among the jars so that each would contain the desired number of larvae of about the same age. The experiments extended from July 1 to November 1, 1927.

[1] Received for publication Mar. 6, 1930; issued September, 1930.
[2] Resigned June 20, 1928.

All nicotine solutions used were made up to the desired molar concentration by diluting 96 per cent nicotine with distilled water. The solutions of nicotine sulphate and nicotine hydrochloride were adjusted to the desired hydrogen-ion concentration with 0.1 normal sulphuric or 0.1 normal hydrochloric acid solutions, respectively, and then diluted. The pH values were determined colorimetrically and checked by the hydrogen electrode, using a saturated calomel half cell, a saturated potassium chloride salt bridge, and a Clark shaking hydrogen electrode assembly with platinum electrodes. The values obtained by the two methods agreed within about 0.1 pH, the electrometric values always being higher.

A group of 10 active, feeding larvae in the last instar was used for each experiment except the tests with 0.1 molar solutions in which the time of action was very short. The larvae were removed from the culture to a small dry beaker by means of a medicine dropper with a wide orifice. The culture solution carried over with the larvae was then removed until not more than about 0.1 c. c. remained in the beaker. Thirty-five cubic centimeters of the nicotine solution at the temperature of the water bath were gently poured into the beaker, which was then placed in a water bath maintained at $26 \pm 1°$ C. A few experiments were made at slightly lower temperatures, but the limits for the entire series fell between 24° and 27°. The volume of the solution stated above was not rigidly adhered to, and sometimes was as low as 12 c. c. Since 10 larvae of the size used in these experiments weigh about 32 mgm., the quantity of nicotine solution was always very large as compared with the mass of the larvae.

The time to the nearest second from the immersion of the larvae in the nicotine solution to the time the fifth one of the 10 became immobile was taken as the time of action of the particular nicotine concentration for 50 per cent of the larvae in each experiment. The action of 0.1 molar nicotine solution was so rapid that it was necessary to reduce the number of larvae in each experiment to four.

THE PROCESS OF POISONING

The course of poisoning of Culex larvae in a nicotine solution requires some consideration at this point. In a 0.03 molar solution the larvae swim about for a brief period, making few excursions to the surface. Soon, however, they fall writhing to the bottom of the beaker, and at times make short, vigorous, uncoordinated swimming movements. Movement gradually subsides, and the body then stiffens into a state of comparative immobility. This was chosen as the end point, and it is quite decisive to an experienced worker. Slight twitching, especially of the siphon, still occurs in response to gentle pressure with a needle, the dorsal heart continues to pulsate, and movements of the digestive tract are visible through the translucent body wall.

The effects of other concentrations differ essentially from the one just described only in the time of appearance of the characteristic phenomena. Life continues for a long, but variable, period following the beginning of this immobile stage. It is manifested by feeble body movement, irregular heart action, and movement of the digestive tract. Preliminary experiments have shown a certain degree

of recovery following transference to water after the first immobile stage is reached, but a quantitative study of it has not yet been completed.

Theoretically a compound like nicotine may enter the body of a submerged Culex larva by at least four routes: (1) Through the siphon and thence into the tracheal system; (2) through the tracheal gills on the anal segment into the tracheal system; (3) through the cuticula into the tissues and blood; (4) through either the mouth or anus into the digestive tract.

The siphon opening of the Culex larva is guarded by five petallike valves which are usually held tightly together when the larva is submerged but which open when the tip of the siphon is pressed against the surface film as the larva seeks a fresh supply of oxygen. Although Wesenberg-Lund (*24, p. 21*)[3] observed that the valves are not always completely closed when the normal larva is submerged in water, frequent observations made by the writers showed that it was closed when the larva was placed in the nicotine solution. It is therefore believed that little if any nicotine, either as gas or in solution, enters the body through the siphon.

Structurally, the tracheal gills appear to offer an excellent opportunity for the passage of substances dissolved in water. However, no evidence that the gills are permeable to nicotine was obtained from this study.

The chitinous cuticula of the larva is probably at most slowly permeable to nicotine. Indirect evidence of its impermeability to this compound is furnished by experiments with Culex pupae, which survive for long periods (20 hours or more) in nicotine solutions (0.003 M and 0.012 M) and may even transform to adults before death. The pupae have no mouth opening. In view of the lack of evidence to the contrary, the writers believe that the toxic effects of nicotine in solution produced in Culex larvae are not due to the passage of the compound through the chitinous cuticula.

When first placed in a weak solution of nicotine the larva swims about, making typical feeding movements with the rotary mouth brushes. As soon as the effects of the poison become apparent, the larva opens the mandibles widely. Under these conditions it appears certain that the nicotine enters the digestive tract in quantities sufficient to produce toxic effects. Entrance through the anus is also a possibility, but evidence in support of it is lacking.

From a consideration of the structure of the larvae and their behavior in the solutions, it is probable that nicotine enters the body chiefly through the mouth as molecules or ions in solution rather than as molecules in the gaseous condition. Bodine (*1*) concluded that certain acids and mercuric chloride penetrate mosquito larvae orally rather than cutaneously.

EXPERIMENTAL RESULTS

THE TOXICITY OF NICOTINE AND NICOTINE SULPHATE SOLUTIONS AT VARIOUS pH VALUES

Nicotine solutions of 0.03 molar concentration, titrated with 0.1 normal sulphuric acid solution to pH values from 2.4 to 7.0, were used in a series of experiments on mosquito larvae. The results are

[3] Reference is made by number (italic) to Literature Cited, p. 347.

summarized in Table 1, and are plotted in Figure 1 as reciprocals of the time in seconds to immobility of 50 per cent of the larvae. The toxic effect of the solutions changes at first slowly with rising pH value, then more abruptly, attaining a maximum for the solution of highest pH value, i. e., nicotine base. Similar results were obtained with solutions titrated to the desired pH with hydrochloric acid solution (Table 1), indicating that the toxicity of the various solutions was not due to a specific action of the sulphate ion.

TABLE 1.—*Toxicity of nicotine, nicotine sulphate, and nicotine hydrochloride solutions at various pH values to the larvae of Culex pipiens in 30 groups of 10 larvae for each test, 1927*

Compound	Molar concentration of nicotine	pH	Dates of experiments	Mean seconds to immobility of 50 per cent of larvae [a]	Coefficient of variation [b]
Nicotine sulphate	0.03	2.4	Sept. 16, 20	2,425±65	14.7
Do	.03	3.6	Sept. 17, 20	2,122±78	20.1
Do	.03	5.0	Sept. 14, 20	1,340±43	17.3
Do	.03	7.0	Aug. 16, 17; Sept. 21	507±37	39.6
Nicotine	.03	9.7	Sept. 13, 15, 21	321±15	24.9
Nicotine hydrochloride	.03	3.6	Oct. 27, 28, 29	1,882±77	22.4
Do	.03	5.0	Oct. 4, 5, 26	1,363±49	19.5

[a] The values for the error of the mean (σ_M) were computed from the formula: $\sigma_M = \frac{\sigma}{\sqrt{N}}$, in which σ is the standard deviation and N the number of groups of larvae.

[b] The coefficient of variation is the ratio (σ : mean seconds to immobility) multiplied by 100.

The curves for toxicity of nicotine to mosquito larvae and for the dissociation of nicotine are compared in Figure 1. The dissociation constants for a 0.01 molar solution at 15° C. and the pH values of the neutral and basic salts of nicotine are taken from Kolthoff (*9*, *10*). Nicotine is a weak diacid base. The first dissociation constant is 7.07×10^{-7} ($pK_1 = 6.16$); the second dissociation constant is 1.12×10^{-11} ($pK_2 = 10.96$). The first dissociation apparently involves the nitrogen of the pyrrolidine ring, the basic salt having a pH value of 5.6 whereas the second dissociation involves the nitrogen of the pyridine ring, the pH value of the neutral salt being 2.6. The pK value for pyridine, as given by Kolthoff, is 8.90.

An inspection of Figure 1 shows a close relationship between the first dissociation curve for nicotine and the curve for toxicity—both the percentage of dissociation and the speed of toxic action, expressed in reciprocals of the time in seconds elapsing until the larvae become immobile, being plotted against pH. The second dissociation, however, affects the shape of the toxicity curve somewhat, although to a very much less extent than does the first dissociation. As a result, in the region of the pH value of the basic salt (pH 5.6) toxicity approaches its minimum value.

These curves are believed to demonstrate two propositions: (1) That the speed of entrance of nicotine from an aqueous solution into Culex larvae is related to the concentration of undissociated nicotine base in the solution; (2) that in so far as the toxicity is concerned with the ionization of the nicotine molecules, it is governed largely by the dissociation of the pyrrolidine nitrogen. The latter statement is made on the assumption that the first dissociation of nicotine

must be governed by the pyrrolidine nitrogen in the same way that the second dissociation is governed by the dissociation of the pyridine nitrogen. The pK value of methylpyrrolidine has apparently not been determined but will probably be found to be less than 6.

THE TOXICITY OF NICOTINE AND NICOTINE SULPHATE SOLUTIONS AT DIFFERENT CONCENTRATIONS OF NICOTINE

Nicotine solutions of 0.1, 0.03, 0.01, and 0.001 molar concentrations were each titrated to pH 5.0 with 0.1 N sulphuric acid solution and compared in toxicity with solutions of the base at the same molar concentrations. The results are given in Table 2 and Figure 2.

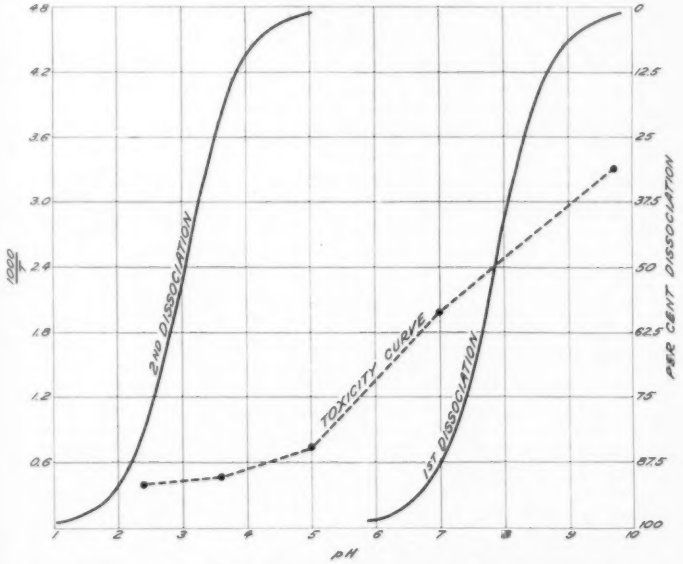

FIGURE 1.—Toxicity to the larvae of *Culex pipiens* of 0.03 M nicotine at various hydrogen-ion concentrations compared with the dissociation of nicotine. The toxicity is plotted in terms of the reciprocals of the time *T*, to the seconds needed to produce immobility in the larvae. The dissociation curves are taken from the works of I. M. Kolthoff (*9*, *10*). This figure shows the two series of data by superimposed graphs. The figures for the toxicity curve are shown at the left of the chart and the percentages for the dissociation curves at the right

The speed of toxic action of the nicotine base, expressed as the reciprocal of the time in seconds to immobility, follows nearly a straight line between the concentrations of 0.01 and 0.1 molar. In this range the proportion of nicotine ions falls from about 3 to less than 1 per cent. The increase in toxicity with increasing concentration is therefore chiefly due to the effect of the content of undissociated nicotine hydroxide molecules rather than to the nicotine-ion content. In the solutions containing nicotine sulphate (pH 5.0), more than 99 per cent of the molecules are in the ionic state, and the speed of toxic action for them at every concentration is much less than for the solutions of the base having the same nicotine concentration. When the ratio (seconds to immobility in the nicotine

TABLE 2.—*Toxicity of nicotine and nicotine sulphate solutions to the larvae of Culex pipiens at various base concentrations*

[Nicotine sulphate solutions adjusted to pH 5.0]

Compound	Molar concentration of nicotine	pH	Dates of experiments	Number of larvae in each group	Number of groups	Mean seconds to immobility of 50 per cent of larvae	Coefficient of variation [a]	Mean ratio of toxicities of base and salt
Nicotine	0.1	9.95	Oct. 8, 10, 11, 21, 24, 26	4	49	44±1.8	29.3	6.7
Nicotine sulphate	.1	5.0	Oct. 8, 6, 7, 21, 24, 26	4	49	281±14	35.9	
Nicotine	.03	9.7	Sept. 13, 15, 21	10	30	321±15	24.9	4.5
Nicotine sulphate	.03	5.0	Sept. 14, 20	10	30	1,350±43	17.3	
Nicotine	.03	9.7	July 20, 21, 22, 23, 25, 27, 28, 29; Aug. 2.	1	200	176±5	43.8	6.8
Nicotine sulphate	.03	5.0	July 23, 25, 26, 27, 28, 29; Aug. 1, 2.	1	200	992±51	72.9	
Nicotine	.01	9.6	Sept. 7, 8, 9, 10	10	20	1,622±54	14.9	6.2
Nicotine sulphate	.01	5.0	Sept. 9, 10	10	20	9,783±142	6.5	
Nicotine	.001	8.5	Sept. 1, 6	10	20	4,056±309	34.1	[b]6.2
Nicotine sulphate	.001	5.0	Sept. 7, 8	10	20	[b]25,200		

[a] The coefficient of variation is the ratio (σ: mean seconds to immobility) multiplied by 100.
[b] Estimated.

sulphate to seconds to immobility in the base) is calculated for each group of larvae—the groups in each series of tests being arranged in pairs in chronological order of the experiments—the mean of all of the ratios tends toward values between 5 and 7; i. e., nicotine base at the concentrations used was about 5 to 7 times more toxic than nicotine sulphate at pH 5.0. This result was obtained with individual larvae as well as with groups of 10 larvae. (Table 2.)

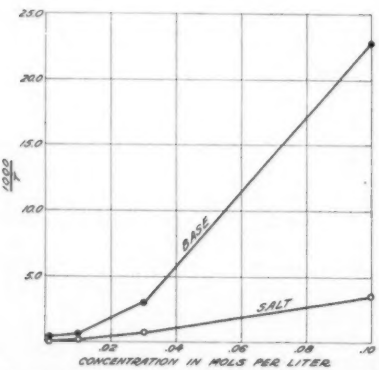

FIGURE 2.—Comparison of the toxicity of nicotine and nicotine sulphate (pH 5.0) to the larvae of *Culex pipiens* at different molar concentrations. The toxicity is plotted in terms of the reciprocals of the time, *T*, in seconds needed to produce immobility of the larvae

THE EFFECT OF SUPPRESSING THE IONIZATION OF NICOTINE BY THE ADDITION OF HYDROXYL IONS

Since an increase in the concentration of unionized nicotine increases the toxicity of nicotine solutions, the question arises if toxicity can also be increased when the ionization of the nicotine base is suppressed by the addition of hydroxyl ions. However, the nicotine base is so slightly ionized that suppression of the ionization would be expected to yield very little additional toxicity. For instance, a solution containing 0.03100 mols of the nicotine base per liter theoretically contains 0.03085 mols of unionized nicotine per liter, solutions of 0.01000 molar strength contain 0.00992 mols of unionized nicotine per liter, and those of 0.001000 molar strength contain 0.000974 mols of unionized nicotine per liter. When these solutions are made to contain 0.01 mols of sodium hydroxide per liter the ionization of nicotine may be said to be completely suppressed, since in any of these

solutions there was less than 1 part in a thousand of nicotine that remained ionized. The suppression of ionization by the sodium hydroxide, therefore, only affected 4.7 parts in a thousand in the case of 0.031 molar nicotine, 8.4 parts in that of 0.01 molar nicotine, and 26 parts in that of 0.001 molar nicotine.

Solutions of 0.001 M, 0.01 M, and 0.03 M nicotine were made up to contain from 0.001 M to 0.05 M sodium hydroxide, the excess of hydroxyl ions being sufficient to suppress almost completely the ionization of the nicotine. The results are expressed in Figure 3 as ratios of the time in seconds needed to bring about immobility in nicotine solution containing sodium hydroxide to time in seconds to the same effect in nicotine alone.

Solutions of sodium hydroxide of a concentration of 0.05 M or less are comparatively nontoxic to Culex larvae, the larvae dying only after exposure to such solutions for from 2 to 12 hours.

The results with 0.03 M and 0.001 M nicotine show clearly that a further suppression of ionization of the nicotine leads to no significant change in toxicity, the ratio, 1.0, being closely approached in both cases. The values obtained for 0.01 M nicotine are, with the one exception shown, more irregular.

A series of experiments in which barium hydroxide, calcium hydroxide, and potassium hydroxide were substituted for sodium hydroxide gave toxicity values in good agreement with those for sodium hydroxide.

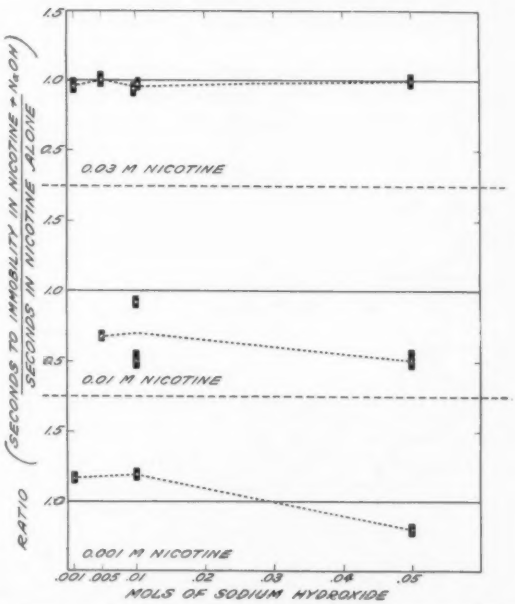
RATIO (SECONDS TO IMMOBILITY IN NICOTINE + NaOH / SECONDS IN NICOTINE ALONE)
1.5
1.0
0.5
0.03 M NICOTINE
0.01 M NICOTINE
0.001 M NICOTINE
.001 .005 .01 .02 .03 .04 .05
MOLS OF SODIUM HYDROXIDE

FIGURE 3.—Relation of the suppression of ionization of nicotine by sodium hydroxide to the toxicity to larvae of *Culex pipiens*. Along the ordinate are indicated ratios of seconds needed to produce immobility in the larvae in nicotine with sodium hydroxide to time in seconds to produce the same in nicotine alone. Along the abscissa are indicated mols of sodium hydroxide added to the nicotine solutions

The conclusion reached may be stated as follows: In aqueous nicotine solutions of 0.001 M concentration or higher, the addition of an inorganic base increases the concentration of unionized nicotine so slightly that under these conditions no increase in toxicity can be observed.

THE TOXICITY OF PYRIDINE AND METHYLPYRROLIDINE

Pyridine solutions of 0.03 and 0.12 molar concentrations were titrated to pH 5.0 and 4.9, respectively, with 0.1 normal sulphuric acid, and their toxicities to Culex larvae were studied in comparison with solutions of pyridine base of the same concentration. A few experiments were also made with a small sample of methylpyrrolidine,[4] the concentrations being 0.03 M, the pH values for the base approximately 9.8, and for the salt (hydrochloride) 3.0.

The results are given in Table 3. They show clearly that these compounds, components of the nicotine molecule, are more toxic as bases than as salts. Both compounds, either as bases or salts, are strikingly less toxic than nicotine, indicating that the toxicity of nicotine results from something inherent in the combination of pyridine with methylpyrrolidine rather than in either of its components.

TABLE 3.—*Toxicity of pyridine and methylpyrrolidine (base and salt) to the larvae of Culex pipiens*

Compound	Molar concentration	pH	Dates of experiments	Number of larvae in each group	Number of groups	Mean seconds to immobility of 50 per cent of larvae	Coefficient of variation [a]	Mean ratio of toxicities of base and salt
Pyridine	0.12	7.8	Aug. 10, 11	1	40	702±26	23.5	2.2
Pyridine sulphate	.12	4.9	Aug. 12	1	40	1,566±56	22.6	
Pyridine	.03	7.1	Nov. 1	10	10	2,048±81	12.5	2.2
Pyridine sulphate	.03	5.0	Nov. 3	10	10	4,432±34	2.4	
Methylpyrrolidine	.03	9.8	Nov. 4	10	4	2,895±106	7.3	[b] 18.6
Methylpyrrolidine hydrochloride	.03	3.0	Nov. 4	10	6	[b] 54,000		

[a] The coefficient of variation is the ratio (σ: mean seconds to immobility) multiplied by 100.
[b] Estimated.

DISCUSSION

The idea that nicotine is more effective against insects than its salts seems to be an old one. In 1900, Del Guercio (*6*) reported some experiments in which silkworms (*Bombyx mori* L.) were sprayed with aqueous solutions of nicotine, nicotine containing alkali, and nicotine containing acid. No differences were noticed in the effects produced by the solution of nicotine and the nicotine containing alkali, but the acidulated nicotine was less active than the other solutions. Vermorel and Dantony (*23, p. 23*) added sodium carbonate to spray solutions containing a nicotine salt, explaining that its purpose was to liberate the nicotine from the combination. They further stated that it has been established for a long time that free nicotine is more toxic than nicotine salts. Hollister (*7*) submerged bedbugs (*Cimex lectularius* L.) in solutions of nicotine and nicotine sulphate of various concentrations, and claimed a slightly higher toxicity for the nicotine solutions. McIndoo (*15*) published the first extensive work on the action of nicotine as an insecticide. He concluded that nicotine spray solutions neither pass into the tracheae nor penetrate the integument, but that nicotine vapor enters the body through the tracheae and passes thence to the tissues. He employed solutions of nicotine and of nicotine sulphate but reported

[4] The sample of methylpyrrolidine was kindly furnished by F. B. LaForge of the Insecticide Division, Bureau of Chemistry and Soils.

no difference in their action on insects. Moore and Graham (*17*) compared the effect of a solution of nicotine sulphate with a nicotine sulphate solution rendered alkaline with sodium carbonate upon the aphid *Macrosiphum sanborni* Gillette, the solutions being applied as sprays. The nicotine solution containing sodium carbonate was considerably more active than the nicotine sulphate solution without this addition. The results were held to be due to the presence of nicotine in volatile form in the alkaline solution. In a later paper (*18*) the same writers, confirming McIndoo's results, showed that nicotine solution does not penetrate into the tracheae but that nicotine vapor does enter them. De Ong (*4, 5*) sprayed and fumigated aphids with 0.1 per cent solutions of nicotine sulphate of various pH values from 6.5 to 8.2. The results from the two methods of application were parallel, the toxicity increasing with the increase of the pH value of the solutions. He believes that nicotine is a tracheal rather than a true contact insecticide. Worthley (*25*) has recently confirmed previous work in showing that alkalies increase the toxicity of nicotine sulphate solutions.

A few references to the relative toxic effect of nicotine as a base and as a salt on other animals than insects have been found in the literature. In 1889, Langley and Dickinson (*12, p. 426*) noted that if a nicotine solution were neutralized with sulphuric acid solution its effect both upon nerve fibers and upon ganglion cells was lessened. No explanation of these observations was attempted. Moore and Row (*16*) studied the comparative physiological action of nicotine, piperidine, and coniine upon the frog. They found that the free bases were more active than the respective salts, 2 mgm. of basic nicotine being as active as 10 mgm. of nicotine hydrochloride. De Ong (*5*) showed that free nicotine, administered orally, was very much more toxic to chickens than nicotine sulphate.

Studies on the toxicity of other alkaloids and similar compounds in relation to the reaction of the dissolving medium have been numerous, dating back at least as far as 1897, when Overton (*19, p. 208*) showed that ammonia, various amines, and many alkaloids enter cells readily as free bases, whereas the salts of these compounds if dissociated do not penetrate cells to a noticeable degree. Crane (*3*) found that the toxicity of alkaloids to *Paramoecium caudatum* varied with the dissociation constant of the base and stated that the free, undissociated base is apparently responsible for the toxicity. No evidence of a direct action of the hydrogen ions upon the resistance of the cell was obtained. Labes (*11*) studied the effect of hydrogen-ion concentration upon the toxicity of certain alkaloids and acids to bacteria and frog larvae and concluded that the undissociated, lipoid-soluble base was more toxic than the lipoid-insoluble ions. Copeland and Notton (*2*) found that the action of a local anaesthetic depends upon the specific selective affinity of its base for nerve fibrils and that the different salts of these compounds vary in their action according to the degree of dissociation. Anaesthetic action is relatively high when the base is combined with a weak acid, the solution having a high pH value, and is relatively low when it is combined with a strong acid, the pH value then being lower. Trevan and Boock (*22*) have studied the relation of hydrogen-ion concentration to the action of a number of local anaesthetics on the rabbit's cornea. The results are held to be consistent with the view that the free base is the only

active constituent of the solutions, the ions or undissociated salt molecules taking no part.

The investigations of the writers referred to above indicate, in general, that nicotine base is more toxic than nicotine salt when administered to insects as a spray or dip, and that it is more toxic to vertebrates as the free base when administered either by mouth or by injection. The results of studies on other alkaloids and organic bases, in which a variety of organisms was used, are in accord with those on nicotine.

The greater insecticidal effect of nicotine as compared with nicotine sulphate has been attributed to the action of nicotine gas molecules which are able to enter and diffuse through the tracheal system. The present studies show, however, that the free base in aqueous solution may also be more toxic to insects than the salt.

It should be noted that nicotine solutions of pH 2.4 still possess an appreciable toxicity although less than that of the free base. This apparently signifies that the ions are, in themselves, somewhat toxic, for in solutions having the concentrations of those used in this study ionization of the nicotine salt molecules is nearly complete.

Although the penetration of gases into living cells is a familiar phenomenon (cf. Jacobs, *8, p. 133*), it seems doubtful whether nicotine can long remain in this state within the tracheal system of an insect because of its great affinity for water. It therefore probably enters the cell as molecules or ions in solution. Lillie (*13, 14*), working with certain organic acids which produce their maximum effect as undissociated molecules, holds that the acid molecules penetrate cell membranes rapidly and dissociate within the cell, yielding acid ions. Taylor (*21, p. 218*), who also worked with acids, believes that the hydrogen ions of the acid are adsorbed into the cell membrane, and the electric charge on the membrane then attracts the acid anion, the pair of ions gradually passing into the cell. It is not impossible that nicotine reacts with certain constituents of cells. The recent work of Petrunkin and Petrunkin (*20, p. 108*), in which they express the belief that certain alkaloids and organic bases combine with gelatin and brain proteins only on the alkaline side of the isoelectric point, are of interest in this connection.

SUMMARY

The toxicity of nicotine to the house mosquito (*Culex pipiens* L.) was studied in aqueous solutions at various pH values. A few similar experiments were also made with pyridine and methylpyrrolidine.

Solutions of 0.03 M concentration adjusted to pH values of 2.4, 3.6, 5.0, and 7.0, with sulphuric acid and at pH 9.7 (the free base) showed a toxic action that increased with increasing pH value. Solutions adjusted to pH 3.6 and 5.0 with hydrochloric acid gave similar results to those adjusted with sulphuric acid.

Solutions of 0.1 M, 0.03 M, 0.01 M, and 0.001 M concentrations were adjusted to pH 5.0 with sulphuric acid and compared in toxicity with solutions of the base of the same molar concentrations. At each nicotine concentration, the free base is about 5 to 7 times more toxic than is nicotine sulphate solution at pH 5.0.

The addition of an inorganic hydroxide (0.05 to 0.001 N) to an aqueous solution of nicotine is without apparent effect upon the toxicity of that solution.

Pyridine solutions of 0.03 M and 0.12 M were about twice as toxic as solutions of the same molar concentration titrated to pH 5.0 and 4.9, respectively, with sulphuric acid. Methylpyrrolidine solution (0.03 M) was about 19 times more toxic than methylpyrrolidine hydrochloride solution of pH 3.0.

The speed of toxic action to the larva of *Culex pipiens* of nicotine, pyridine, and methylpyrrolidine in aqueous solution is directly related to the concentration of the undissociated molecules.

It is believed that toxicity results largely from the penetration of the molecules into the body through the wall of the alimentary tract. Nicotine ions are somewhat toxic but much less so than nicotine molecules. It is also believed that the change in toxicity of a nicotine solution with change in pH results largely from the dissociation of the pyrrolidine nitrogen.

Previous writers have explained the greater toxicity of nicotine over nicotine sulphate on the basis of the greater volatility of the former. In this study it is shown that the free base in solution is also much more toxic than nicotine sulphate.

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